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G01N 33/566, 33/50, C07K 15/06, C07D 339/08, 339/06, 327/04, 319/06, 317/30, 409/04, 333/38, C07C 63/49, 63/66, 65/36, A61K 31/335, 31/19

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(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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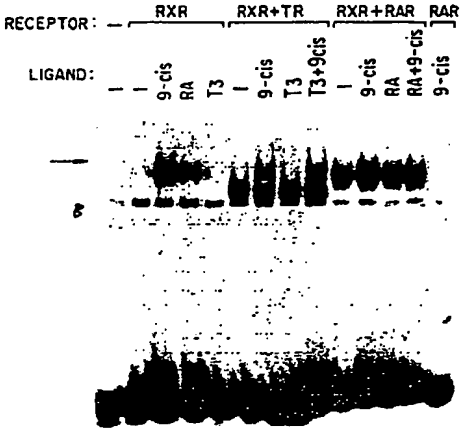
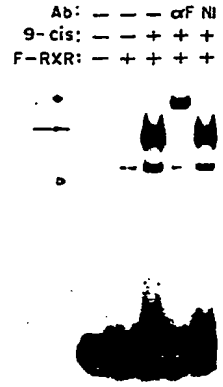
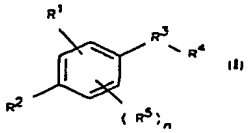
29 September 1994 (29.09.94)

(54) Title: RXR HOMODIMER FORMATION AND BRIDGED BICYCLIC AROMATIC COMPOUNDS AND THEIR USE IN MODULATING GENE EXPRESSION







(57) Abstract

The invention provides a method of screening a substance for the ability to affect the formation of a retinoid X receptor homodimer comprising combining the substance and a solution containing retinoid X receptors and determining the presence of homodimer formation. The screening method can be used to determine compounds which selectively activate homodimer formation and heterodimer formation. Also provided is a method of screening a substance for an effect on a retinoid X receptor homodimer's ability to bind DNA comprising combining the substance with the homodimer and determining the effect of the compound on the homodimer's ability to bind DNA. Finally, the invention provides methods of activating retinoid X receptor homodimer formation. Bridged bicyclic aromatic compounds are provided having structure (I); wherein R^1, R^2, R^3, R^4, R^5 and n are as defined herein. The compounds are useful for modulating gene expression of retinoic acid receptors, vitamin D receptors and thyroid receptors. Pharmaceutical compositions and methods for modulating gene expression are provided as well.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US 93/11492

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 G01N33/566 G01N33/50 C07K15/06 C07D339/08 C07D339/06
C07D327/04 C07D319/06 C07D317/30 C07D409/04 C07D333/38
C07C63/49 C07C63/66 C07C65/36 A61K31/335 A61K31/19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K G01N A61K C07C C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATURE, vol.358, 13 August 1992, LONDON GB pages 587 - 591 XIAO-KUN ZHANG ET AL. 'Homodimer formation of retinoid X receptor induced by 9-cis retinoic acid' see the whole document ---	1-4,6,7, 12
A	EP,A,0 260 162 (CENTRE INTERNATIONAL DE RECHERCHES DERMATOLOGIQUES C.I.R.D.) 16 March 1988 see claims 1-15,24-31 --- -/--	13-42, 65,66, 69,70

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search
20 April 1994

Date of mailing of the international search report
24 -08- 1994

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INTERNATIONAL SEARCH REPORT

Internation pplication No
PCT/US 93/11492

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	SCIENCE, vol.258, 18 December 1992 pages 1944 - 1946 JÜRGEN M. LEHMANN ET AL. 'Retinoids selective for retinoid X receptor response pathways' see the whole document	1-4,6,7, 12-17, 19,20, 25-28, 32-34, 37,43, 46,47
E	WO,A,93 25223 (LA JOLLA CANCER RESEARCH FOUNDATION) 23 December 1993	1-7,12, 43,44, 46,49, 50,54, 57,58, 61,64

3

INTERNATIONAL SEARCH REPORT

Int .tional application No.
PCT/US 93/ 11492

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 54-60, 67-70 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see annex

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-7,12-50,54-70

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1.- claims 1-7,12-50,54-70: Method for screening a substance for the ability to affect RXR homodimer formation. Substances thereof and their uses to affect homodimer formation, and compositions containing them. Purified RXR homodimers.

2.- claim 8: Method for screening a substance which induces RXR heterodimer formation.

3.- claim 9: Method for screening a substance which affects RXR homdimer binding to DNA.

4.- claims 10,11,51,52: Method for screening response elements for binding to RXR homodimers.

5.- claim 53: Method for detecting a pathology associated with RXR homodimer formation.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internatio Application No
PCT/US 93/11492

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0260162	16-03-88	FR-A-	2601670	22-01-88
		AU-B-	597396	31-05-90
		AU-A-	7590387	04-02-88
		CA-A-	1328605	19-04-94
		ES-T-	2002463	16-01-94
		JP-A-	63030433	09-02-88
		US-A-	4833240	23-05-89
WO-A-9325223	23-12-93	AU-B-	4636893	04-01-94

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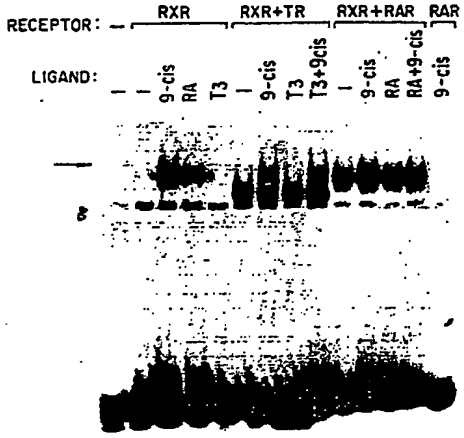
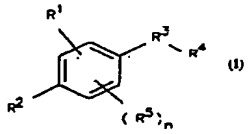




(57) Abstract

The invention provides a method of screening a substance for the ability to affect the formation of a retinoid X receptor homodimer comprising combining the substance and a solution containing retinoid X receptors and determining the presence of homodimer formation. The screening method can be used to determine compounds which selectively activate homodimer formation and heterodimer formation. Also provided is a method of screening a substance for an effect on a retinoid X receptor homodimer's ability to bind DNA comprising combining the substance with the homodimer and determining the effect of the compound on the homodimer's ability to bind DNA. Finally, the invention provides methods of activating retinoid X receptor homodimer formation. Bridged bicyclic aromatic compounds are provided having structure (I); wherein R^1, R^2, R^3, R^4, R^5 and n are as defined herein. The compounds are useful for modulating gene expression of retinoic acid receptors, vitamin D receptors and thyroid receptors. Pharmaceutical compositions and methods for modulating gene expression are provided as well.

RXR HOMODIMER FORMATION AND BRIDGED BICYCLIC AROMATIC COMPOUNDS AND THEIR USE IN MODULATING GENE EXPRESSION

TECHNICAL FIELD

This invention relates generally to the regulation of gene expression by retinoid receptors, and more particularly relates to the retinoid x receptor and novel bridged bicyclic aromatic compounds that are useful in modulating gene expression by retinoic acid receptors, retinoid X receptors, vitamin D receptors and thyroid receptors.

BACKGROUND ART

The vitamin A metabolite all-trans-retinoic acid (RA) and its natural and synthetic derivatives (retinoids) exert a broad range of biological effects[1,2]. Clinically, retinoids are important therapeutics in the treatment of skin diseases and cancers[3-6]. Understanding how the multitude of retinoid actions can be mediated at the molecular level has been greatly enhanced by the cloning and characterization of specific nuclear receptors, the retinoic acid receptors (RARs)[7-12] and the retinoid X receptors (RXRs)[13-17]. RARs and RXRs are part of the steroid/thyroid hormone receptor superfamily[18,19]. Both types of receptors are encoded by three distinct genes, α, ß, and γ, from which, in the case of RARs, multiple isoforms can be generated[20-22]. Interestingly, while RARs are specific to vertebrates, the RXRs have been well conserved from Drosophila to man[17,23]. Despite the considerable advances in the understanding of the molecular mechanisms of retinoid receptor action in recent years, a central question of whether distinct molecular pathways for naturally occurring retinoids exist has not yet been answered. The recent observation that the RA stereoisomer 9-cis-RA binds with high affinity to RXRs[23,24] suggested a retinoid response pathway distinct from that of all-trans-RA. However, it was almost simultaneously discovered by several laboratories that RARs require interaction with auxiliary receptors for effective DNA binding and function and that RXRs are such auxiliary receptors[15,16,26-29]. Hence, RARs appear to function

effectively only as heterodimeric RAR/RXR complexes, or in combination with comparable auxiliary proteins that still need to be identified. Similarly, RXRs were shown to require RARs, thyroid hormone receptors (TRs), or Vitamin D_3 receptors (VDRs) for effective DNA binding[15,16,26-29]. Thus, from these DNA binding studies, RXRs appeared to be able to function predominantly if not exclusively as auxiliary receptors, thereby playing a crucial role in generating a high degree of diversity and specificity of transcriptional controls and mediating the highly pleiotropic effects of different hormones by increasing DNA affinity and specificity for at least 3 different classes of ligand-activated receptors.

Contrary to these findings, the present invention provides that RXRs form homodimers. The invention provides that these homodimers effectively bind to specific response elements in the absence of auxiliary receptors and their DNA binding specificity is distinct from that of the RXR containing heterodimers. The invention demonstrates a novel mechanism for retinoid action by which a ligand induced-homodimer mediates a distinct retinoid response pathway. Additionally, ligands are provided which selectively activate RXR homodimer formation.

The present invention also provides a new class of retinoids in the form of bridged bicyclic aromatic compounds as will be described in detail herein. These new compounds are useful for regulating and/or eliciting selective gene expression by receptors in the retinoic acid family, i.e., RARs, RXRs, vitamin D receptors (VDRs), and thyroid hormone receptors (THRs). While not wishing to be bound by theory, it is postulated that the presently disclosed and claimed compounds are effective in modulating gene expression by virtue of their capability of interacting with a receptor protein that binds to the aforementioned receptors. The compounds are also believed to be useful in modulating gene expression by inducing formation of RXR homodimers in addition to RAR-RXR, VDR-RXR and THR-RXR heterodimers.

The novel compounds are thus useful for controlling cellular processes that are regulated by thyroid hormone, vitamin D, and retinoids such as 9-cis-retinoic acid, the natural ligand for RXR. Thus, acne, leukemia, psoriasis, and skin aging, all of which are regulated by retinoic acid, may be treated using the compounds of the invention, as may bone calcification, regulated by vitamin D, and energy levels, regulated by thyroid hormone. Unlike the synthetic retinoids of the prior art, the present compounds are believed to provide for substantially reduced side effects and teratogenicity.

SUMMARY OF THE INVENTION

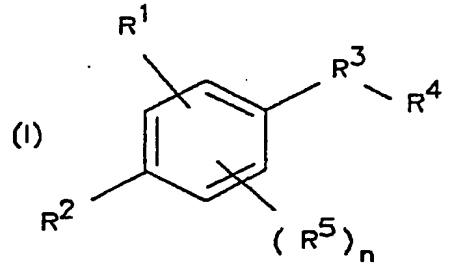
The invention provides a method of screening a substance for the ability to affect the formation of a retinoid X receptor homodimer comprising combining the substance and a solution containing retinoid X receptors and determining the presence of homodimer formation. Also provided is a method of screening a substance for an effect on a retinoid X receptor homodimer's ability to bind DNA comprising combining the substance with the homodimer and determining the effect of the compound on the homodimer's ability to bind DNA. A method of inhibiting an activity of a retinoid X receptor heterodimer comprising increasing the formation of a retinoid X receptor homodimer, thereby preventing the retinoid X receptor from forming a heterodimer and preventing the resulting heterodimer activity is also provided. A method of inhibiting an activity of a retinoid X receptor homodimer is also provided. A method of determining an increased probability of a pathology associated with retinoid X receptor homodimer formation and treating such pathology are further provided. In addition, a method of screening a response element for binding with a retinoid X receptor homodimer is provided. Finally, the invention provides methods of activating retinoid X receptor homodimer formation.

Additionally, it is a primary object of the invention to address the above-mentioned need in the art by providing novel compounds useful to modulate gene expression by a receptor in the retinoic acid family of receptors. It is another object of the

invention to provide novel compounds useful to control cell differentiation processes regulated by thyroid hormone, vitamin D, and/or retinoids such as 9-cis-retinoic acid. It is still another object of the invention to provide novel compounds in the form of bridged, bicyclic aromatic structures. It is yet another object of the invention to provide pharmaceutical compositions containing one or more of the novel compounds. It is a further object of the invention to provide a method for modulating gene expression of a receptor selected from the group consisting of retinoic acid receptors, retinoid X receptors, vitamin D receptors, and thyroid receptors. It is still a further object of the invention to provide a method for controlling cell differentiation processes regulated by thyroid hormone, vitamin D, and/or retinoids such as 9-cis-retinoic acid.

Additional objects, advantages and novel features of the invention will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention.

In one aspect, then, the invention relates to novel compounds having the structural formula (I)



(I)

in which:

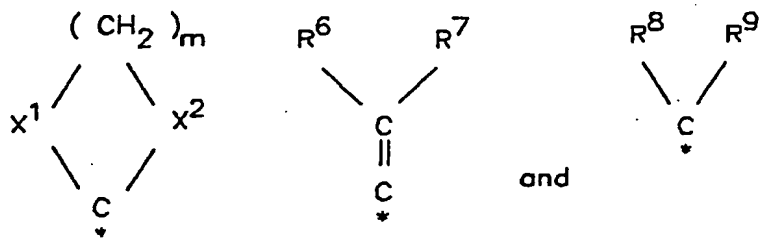
R^1 is selected from the group consisting of lower alkyl and adamantyl;

R^2 is $-O-R^6$ or $-S-R^6$ where R^6 is lower alkyl; or

where R^1 is ortho to R^2, R^1 and R^2 may be linked together to form a 5- or 6-membered cycloalkylene ring, either unsubstituted or substituted with 1 to 4 lower alkyl groups, and optionally containing

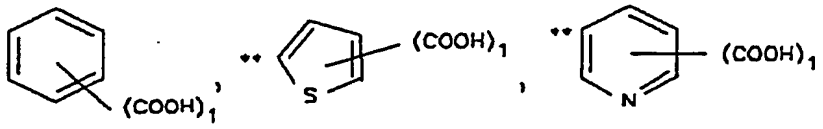
1 or 2 heterocyclic atoms selected from the group consisting of O, S and NR where R is hydrogen or lower alkyl, preferably adjacent to the aromatic ring;

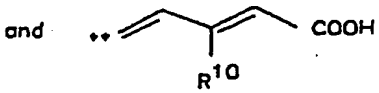
R^3 is selected from the group consisting of carbonyl,



in which X^1 and X^2 are independently selected from the group consisting of O, S and methylene, wherein at least one of X^1 and X^2 is O or S, or wherein one of X^1 and X^2 is NR, and the other is methylene, m is 2 or 3, R^6, R^7, R^8 and R^9 are independently hydrogen or lower alkyl, with the proviso that when n is O, R^6 and R^7 are not both hydrogen and R^8 and R^9 are not both hydrogen, or R^8 and R^9 may be linked together to form a cycloalkylene ring containing 3 to 6 carbon atoms, and * represents the point of attachment of the R^3 substituent to the remainder of the molecule; and

R^4 is selected from the group consisting of





in which R^{10} is hydrogen or methyl, l is 0 or 1, and ** represents the point of attachment of the R^4 substituent to the remainder of the molecule,

the R^5 are independently selected from the group consisting of lower alkyl and lower alkoxy; and

n is 0, 1, 2 or 3,

with the proviso that if n is 0, R^3 is other than carbonyl, $*C=CH_2$ or CH_2.

The invention also encompasses pharmaceutically acceptable esters, amides and salts of such compounds, as will be explained in detail, infra.

In other aspects, the invention relates to pharmaceutical compositions containing the aforementioned compounds and to methods of using the compounds to modulate selective gene expression by a receptor in the retinoic acid family of receptors.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows 9-cis-retinoic acid induces RXR homodimer binding on TREpal.

(a) In vitro synthesized RXRα was incubated either with (+) or without (-) indicated hormones (10^{-7} M 9-cis-RA; 10^{-6} M RA; 10^{-6} M T_3) in the presence or absence of in vitro synthesized TRα or RARβ for 30 min at room temperature. After this preincubation, the reaction mixtures were analyzed by gel retardation assay using ^{32}P-labeled TREpal as probe. Lane 1 represents the nonspecific binding of unprogrammed reticulocyte lysate. Open triangles indicate the nonspecific complex observed with unprogrammed reticulocyte lysate. Solid triangles indicate the specific TRα-RXRα heterodimer binding. Arrows indicate specific RXRα homodimer binding. The RXRα/RARβ heterodimer migrates at the same position as the RXRα homodimer. For comparison, the effect of 9-cis-RA on RARβ binding is shown.

(b) To determine that 9-cis-RA induced DNA binding complex contains RXRα protein, Flag-RXRα (F-RXR), an RXRα derivative that contains an eight-amino-acid epitope (Flag) at its amino terminal end which can be recognized by a specific anti-Flag monoclonal antibody, was constructed. In vitro synthesized F-RXRα was incubated either with (+) or without (-) 10^{-7} M 9-cis-RA in the presence of

either specific anti-Flag antibody (*α*F) or nonspecific preimmune serum (NI) for 30 min at room temperature. The effect of anti-Flag antibody on F-RXR*α* binding in the presence of 9-cis-RA was then analyzed by gel retardation assay using ^{32}P-labeled TREpal as a probe. Lane 1 represents the nonspecific binding of unprogrammed reticulocyte lysate (open triangles). Arrows indicate the specific F-RXR*α* homodimer and RAR-RXR heterodimer binding. Diamonds indicate the anti-Flag antibody up-shifted F-RXR homodimer.

Figure 2 shows the characterization of 9-cis-RA induced RXR homodimer on TREpal.

(a) Cooperative binding of 9-cis-RA induced RXR*α* homodimer. Formation of RXR-DNA complex at different receptor concentrations in the absence or presence of 10^{-7} M 9-cis-RA was analyzed by gel retardation assay using labeled TREpal as probe. Open triangle indicates the nonspecific binding of an unprogrammed reticulocyte lysate. Arrows indicate the specific RXR binding complex.

(b) Quantitation of the RXR binding complex at different receptor concentrations in the presence of 9-cis-RA. Gel slices containing RXR binding complex in the presence of 9-cis-RA shown in Figure 2(a) were excised and counted in a scintillation counter and plotted.

(c) 9-cis-RA concentration-dependent binding of RXR homodimer on TREpal. Equal amounts of *in vitro* synthesized RXR protein were incubated with indicated concentrations of 9-cis-RA. The reaction mixtures were then analyzed by gel retardation assay using labeled TREpal as a probe. Open triangles indicate the nonspecific binding of unprogrammed reticulocyte lysate. Arrows indicate the specific RXR binding complex.

Figure 3 shows 9-cis-RA induces RXR homodimer binding on RXR-specific response elements.

(a) Nuclear receptor binding elements used in this study. These oligonucleotides were synthesized with appropriate restriction sites at both ends as indicated by the small letters. Sequences that are closely related to the AGG/TTCA motif are indicated by arrows.

(b) The effect of 9-cis-RA on RXR homodimer binding of ApoAI-RARE or CRBPII-RARE was analyzed essentially as described in Fig. 1a. Lane 1 represents the nonspecific binding of unprogrammed reticulocyte lysate, which are indicated by the open triangles. Solid triangles indicate the RAR/RXR heterodimer complex. Arrows indicate the specific RXR binding complex.

Figure 4 shows response element specific binding of RXR homodimer. The effect of 9-cis-RA on RXR binding on RA specific response elements (a), T_3 specific response elements (b), or estrogen specific response element (c) was analyzed by gel retardation assays as described in Figure 1a. For comparison, the binding of RXR/RAR heterodimer (a), RXR/TR heterodimer (b) or estrogen receptor (c) is shown. Open triangles indicate the nonspecific binding of unprogrammed reticulocyte lysate. Solid triangle indicates the RAR/RXR heterodimer complex (a), TR/RXR heterodimer complex (b) or ER complexes (c).

Figure 5 shows RXR homodimerization occurs in solution. ^{35}S-labeled in vitro synthesized RXRα proteins were incubated with partially purified bacterially expressed Flag-RXR (F-RXR) (+) or similarly prepared glutathione transferase control protein (-) either in the presence or absence of response elements or chemical cross-linker DSP as indicated. After incubation, either anti-Flag antibody (F) or nonspecific preimmune serum (NI) was added. 10^{-7} M 9-cis-RA was maintained during working process. The immune complexes were washed in the presence of 10^{-7} M-cis-RA, boiled in SDS sample buffer and separated on a 10% SDS-PAGE. The ^{35}S-labeled in vitro synthesized RXRα protein is shown in the right panel.

Figure 6 shows transcriptional activation of RXR and RARα: RXR heterodimers by 9-cis-RA on natural response elements. CV-1 cells

were cotransfected with 100 ng of the reporter plasmids (a) TREpal-tk-CAT (b) βRARE-tk-CAT (c) ApoAI-RARE-tk-CAT and (d) CRBPI-RARE-tk-CAT and 5 ng of empty pECE expression vector, pECE-RXRα, pECE RARα or combination of both as indicated. Transfected cells were treated with no hormone (open bars), 10^{-7} M RA (shadowed bars) or 10^{-7} M 9-cis-RA (dark shadowed bars). The results of a representative experiment performed in duplicate are shown.

Figure 7 shows RXRα-dependent transactivation of reporter constructs (a) TREpal-tk-CAT (10) or (b) CRBPII-tk-CAT (10) by 9-cis-RA or retinoids SR11203, SR11217, SR11234, SR11235, SR11236, and SR11237. Results of a representative experiment carried out four times are shown. In four independent experiments, induction profiles did not vary significantly. CAT activity was normalized for transfection and harvesting efficiency by measuring the enzymatic activity derived from the cotransfected ß-galactosidase expression plasmid (pCH110, Pharmacia).

DETAILED DESCRIPTION OF THE INVENTION

Definitions and Nomenclature:

Before the present compounds, compositions and methods are disclosed and described, it is to be understood that this invention is not limited to specific synthetic methods, specific pharmaceutical carriers, or to particular pharmaceutical formulations or administration regimens, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a bicyclic aromatic compound" includes mixtures of bicyclic aromatic compounds, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

The term "alkyl" as used herein refers to a branched or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like. Preferred alkyl groups herein contain 1 to 12 carbon atoms. The term "lower alkyl" intends an alkyl group of one to six carbon atoms, preferably one to four carbon atoms. The term "cycloalkyl" intends a cyclic alkyl group of three to eight, preferably five or six, carbon atoms.

The term "alkoxy" as used herein intends an alkyl group bound through a single, terminal ether linkage; that is, an "alkoxy" group may be defined as -OR where R is alkyl as defined

above. A "lower alkoxy" group intends an alkoxy group containing one to six, more preferably one to four, carbon atoms.

The term "alkylene" as used herein refers to a difunctional saturated branched or unbranched hydrocarbon chain containing from 1 to 24 carbon atoms, and includes, for example, methylene ($-CH_2-$), ethylene ($-CH_2-CH_2-$), propylene ($-CH_2-CH_2-CH_2-$), 2-methylpropylene [$-CH_2-CH(CH_3)-CH_2-$], hexylene [$-(CH_2)_6-$] and the like. "Lower alkylene" refers to an alkylene group of 1 to 6, more preferably 1 to 4, carbon atoms. The term "cycloalkylene" as used herein refers to a cyclic alkylene group, typically a 5- or 6-membered ring.

The term "alkene" as used herein intends a monounsaturated or di-unsaturated hydrocarbon group of 2 to 24 carbon atoms. Preferred groups within this class contain 2 to 12 carbon atoms. Asymmetric structures such as (AB)C=C(CD) are intended to include both the E and Z isomers. This may be presumed in structural formulae herein wherein an asymmetric alkene is present, or it may be explicitly indicated by the bond symbol ~~~~~.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not. For example, the phrase "optionally substituted phenyl" means that the phenyl may or may not be substituted and that the description includes both unsubstituted phenyl and phenyl where there is substitution.

By the term "effective amount" of a compound as provided herein is meant a nontoxic but sufficient amount of the compound to provide the desired regulation of gene expression. As will be pointed out below, the exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease that is being treated, the particular bicyclic compound used, its mode of administration, and the like. Thus, it is not possible to specify an

exact "effective amount." However, an appropriate effective amount may be determined by one of ordinary skill in the art using only routine experimentation.

By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected bicyclic compound without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

"Eliciting," "modulating" or "regulating" selective gene expression is intended to mean that a compound is capable of acting as an activator or an antagonist of gene expression by a particular receptor, i.e., a receptor in the retinoic acid family.

The "retinoic acid family" of receptors, also termed "retinoid receptors," is intended to encompass retinoic acid receptors, retinoid X receptors, vitamin D receptors and thyroid hormone receptors. The aforementioned three groups of receptors may also be loosely referred to herein as "retinoic acid receptors," i.e., such that the term includes retinoid X receptors, vitamin D receptors and thyroid hormone receptors in addition to retinoic acid receptors themselves.

"Bridged, bicyclic aromatic compounds" intends all compounds encompassed by the structure of formula (I). These compounds may also be termed "retinoids," with that term further intended to include such species as retinoic acid and 9-<u>cis</u> retinoic acid.

The invention provides a method of screening a substance for the ability to affect the formation of an RXR homodimer comprising combining the substance and a solution containing RXRs and determining the presence of a homodimer formation. The presence of homodimer formation can, for example, be determined by detecting the activation of transcription by the RXR homodimer or by coprecipitation. The

affect can be the induction of homodimer formation, for example, an activity similar to that activated by 9-cis-RA or an activity which selectively activates homodimer formation over heterodimer formation. By "selectively activates" is meant a compound which activates homodimer formation but does not significantly activate heterodimers. Alternatively, "selectively activates" includes compounds which activate heterodimer formation but do not significantly activate homodimer formation. "Significant activation" includes activation that is sufficient to cause a harmful pharmological effect on the subject. The affect can also be the inhibition of homodimer formation. Examples of inhibition include a substance which competes for 9-cis-RA binding to the receptor but itself does not activate or induce dimerization or which binds 9-cis-RA to block its activity. Such screening of substances is routinely carried out given the subject discovery of homodimer formation. In particular assays set forth below can generally be used for screening by merely substituting the substance of interest for 9-cis-RA. A good starting point for screening such "substances" is the activity of 9-cis-RA described herein. The substituents on 9-cis-RA can be varied to make 9-cis-RA analogs and screened in the method to determine any increase or decrease in homodimer formation. However, any substance can be screened in this assay to determine any affect on homodimer formation. Such compounds can then be used to promote homodimer formation and gene transcription in a cell. A cell as used herein includes cells found either in vitro or in vivo. Thus, the compounds can be administered to a human subject to effect RXR homodimer formation and promote transcription of a gene activated by an RXR homodimer. Such compounds are set forth in the Examples.

The data set forth herein utilizes RXRα. However, given the high homology between RXRα, β and γ, each protein should form homodimers and have the activity described for RXRα homodimers. Relatedly, homodimers can form between different RXRs. For example, homodimers can form between RXRα and RXRβ or between RXRβ and RXRγ or between RXRα and RXRγ. The activity of these homodimers can be confirmed using the methods set forth herein.

The invention also provides a method of screening a substance for an effect on an RXR homodimer's ability to bind DNA comprising combining the substance with the homodimer and determining the effect of the compound on the homodimer's ability to bind DNA. For example, compounds which might bind the homodimer or bind the DNA response element recognized by an RXR homodimer can be screened in this method.

The invention further provides a method of inhibiting an activity of an RXR-containing heterodimer comprising increasing the formation of an RXR homodimer, thereby preventing the RXR from forming a heterodimer and preventing the resulting heterodimer activity. The activity can be any activity but is generally the activation or repression of transcription. The activity can be blocked, for example, by utilizing RXRs to form homodimers which otherwise would be available to form heterodimers. Since the number of heterodimers are decreased, the activity of the heterodimers is decreased. In one example, the RXR heterodimer is comprised of thyroid hormone receptor and RXR. The activity of the RXR/TR heterodimer was decreased. Other heterodimers can be tested using standard methods given the teaching set forth herein.

The invention also provides a method of inhibiting an activity of an RXR receptor homodimer comprising preventing the formation of the RXR homodimer. Such inhibition can be obtained, for example, by inhibiting 9-cis-RA or the transcription or activation by 9-cis-RA. The activity inhibited is generally the activation or repression of transcription.

The invention also provides a method of inhibiting an activity of an RXR homodimer comprising preventing the binding of the RXR homodimer to its response element. For example, the activity of a receptor which competes for the same response element can be promoted. In general, the activity which is inhibited is the activation or repression of transcription.

The invention still further provides a method of determining an increased probability of a pathology associated with RXR homodimer formation comprising detecting a modulation of RXR homodimer formation in the subject when compared to a normal subject. The modulation can be an increase or a decrease in homodimer formation. Such a modulation can result, for example, from a mutated RXR. The decrease can be detected by an assay for the homodimers in a sample or by detecting mutations known to decrease homodimer formation.

Relatedly, the invention provides a method of treating a pathology associated with RXR homodimer formation in a subject comprising modulating homodimer formation in the subject. The modulation can be an increase or a decrease depending on the pathology. Such an increase can be accomplished, for example, utilizing compounds which promote RXR transcription. The pathology can be associated with the skin, e.g., acne and psoriasis. In addition, the pathology can be a cancer. The exact amount of such compounds required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease that is being treated, the particular compound used, its mode of administration, and the like. Thus, it is not possible to specify an exact activity promoting amount. However, an appropriate amount may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

The invention also provides a purified RXR homodimer. By "purified" is meant free of at least some of the cellular components associated with RXR homodimers in a natural environment.

The invention further provides a method of screening a response element for binding with a RXR homodimer comprising combining the response element with the RXR homodimer and detecting the presence of binding. The presence of binding can be determined by a number of standard methods. In one method, binding is detected by the transcriptional activation of a marker which is operably linked to the

response element. By "operably linked" is meant the marker can be transcribed in the presence of the transcriptional activator.

The invention grows out of our study of the effects of the natural vitamin A derivative 9-cis-RA on retinoid receptor DNA binding and transcriptional activation. In contrast to all-trans-RA, the 9-cis analog dramatically enhances RXRα binding at 10^{-9} to 10^{-8}M concentrations to several RXR-specific RAREs but not to natural TREs or the ERE. The effect is specific to RXR since 9-cis-RA did not induce binding of RARα, β or γ to response elements (Fig. 1, Fig. 3). Judging from the migration pattern in the gel shift assays, we assume that 9-cis-RA induces homodimer formation, although a larger complex containing an RXR trimer or tetramer can occur (particularly in the case of the CRBPII-RARE). Such trimer or tetramer formation can be tested using the methods set forth herein.

RXRα homodimers exert response element specificity distinct from heterodimers. The rCRBPI response element did not interact with RXR homodimers, while the CRBPII response element, the only natural RARE identified so far that contains perfect repeats, was a strong binder of 9-cis-RA induced RXRα homodimers. It has been shown previously that this response element is well activated by RXRα[24,30]. Although this response element is also bound effectively by the RXRα-RARα heterodimer (Fig. 3)[27,29], the heterodimer appears to have a repressor function [30].

The results obtained with the transcriptional activation studies agree well with the DNA binding studies although CV-1 cells, like all other mammalian tissue culture cells tested, contain endogenous retinoid receptors that can partially obscure effects. Nonetheless, response elements that strongly bind 9-cis-RA-RXRα homodimers also responded strongly to cotransfected RXRα in the presence of 9-cis-RA, whereas response elements that did not bind well to RXRα homodimers, like the rCRBPI-RARE or the MHC-TRE, could not be activated by RXRα alone.

It is generally believed that the dimerization - homodimerization or heterodimerization - of nuclear hormone receptors is critical for high affinity interaction of the receptors with their cognate response elements. RXRs exist mainly as monomer in solution[16] and require high concentrations or the presence of RARs, TRs or VDR to display effective DNA binding activity [13,15,16,26-30]. The observation of the enhanced cooperative RXR DNA binding activity in the presence of 9-cis-RA demonstrates that 9-cis-RA induced he formation of RXR homodimers which have an increased affinity for DNA. Thus, binding of 9-cis-RA to RXR caninduce a conformational change, which allows homodimerization to occur. It is interesting that although 9-cis-RA and RA can bind to RAR,[24,25] they do not induce RAR homodimer formation.

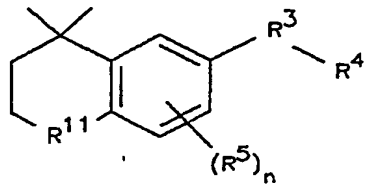
RXRα homodimer formation can occur in solution in the absence of DNA. Thus, when 9-cis-RA becomes available to cells, the equilibrium between monomeric and dimeric receptors is changed and an additional species, the RXR homodimer can be formed, allowing for novel response pathways. The concept of ligand-induced homodimer binding as observed by in vitro gel shift assay has not been previously observed for nuclear receptors with the exception of a mutated estrogen receptor (ER-val-400)[44,45].

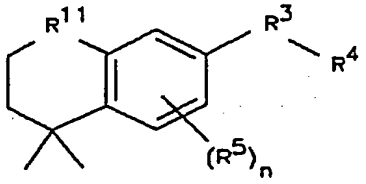
For the related TRs, a ligand effect has been reported on homodimer binding, [46,47] however the ligand (T3) reduced homodimer response element interaction. Since the carboxy terminal half of TRs and RARs encodes both ligand as well as dimerization functions[36,46,48], the strong effect of the ligand on dimerization as observed here is not completely surprising. However, the specificity of the effect is quite dramatic since only homodimer but not heterodimer formation appears to be affected. An overall picture emerges where the carboxy terminal region of a receptor through its intermixed domains (that also includes a transcriptional activation region)[49] allows for multiple activities of individual receptors that may also include interactions with other regulatory proteins[50].

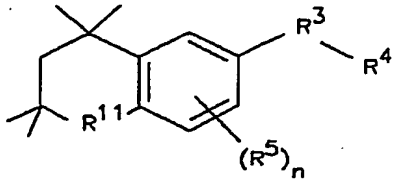
The data presented in this application clearly demonstrate the central role of the RXRs, having dual functions that allow them to

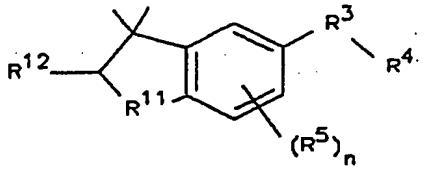
act as auxiliary receptors for three classes of hormone receptors, the RARs, TRs and VDRs through heterodimerization. The two functions of RXR - homodimerization and heterodimerization - represent two distinct transcriptional regulatory controls that can be expected to affect distinct physiological processes. Thus, 9-cis-RA can have therapeutic properties distinct from that of all-trans-RA.

The novel compounds provided herein are those defined by structural formula (I) above. Preferred compounds within this generic structure include

(II) 

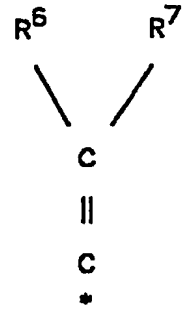
(III) 

(IV)  and

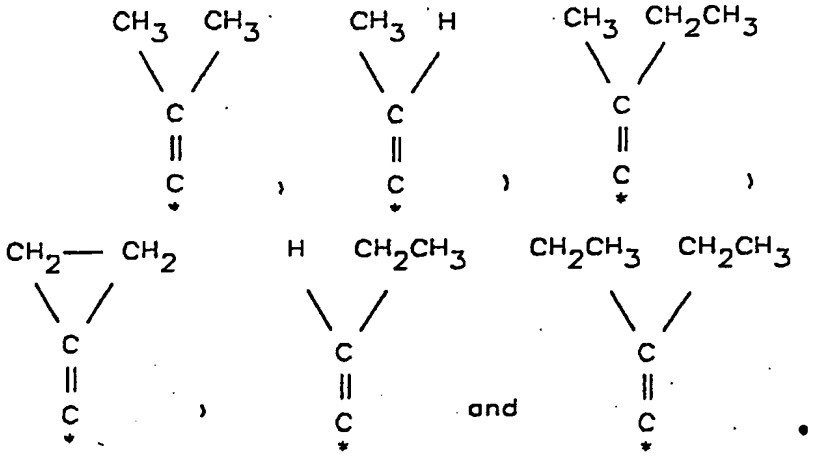
(V) 

where R^{11} is selected from the group consisting of O, S, $(CH_3)_2C$ and CH_2, and R^{12} is hydrogen or methyl. Particularly preferred compounds within this group are as shown in structural formula (II).

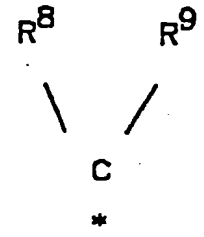
Preferred R^3 substituents, when R^3 has the general structure



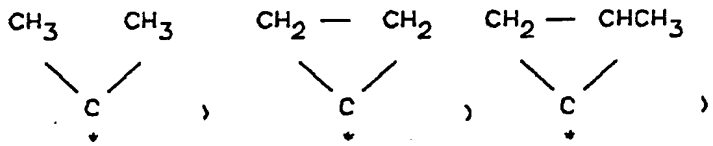
are selected from the group consisting of

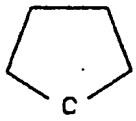


Preferred R^3 substituents, when R^3 has the general structure



are selected from the group consisting of





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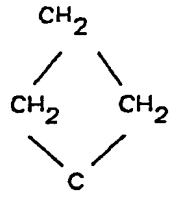
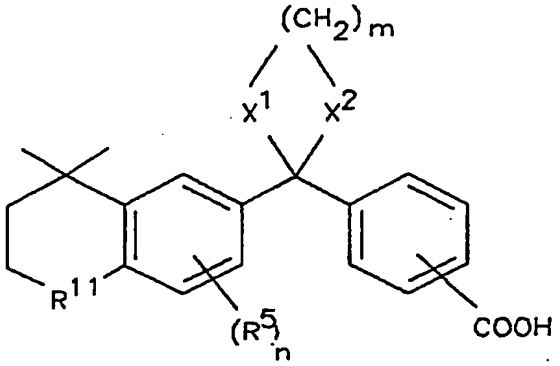
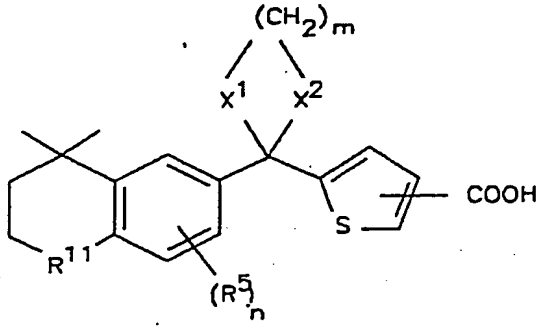
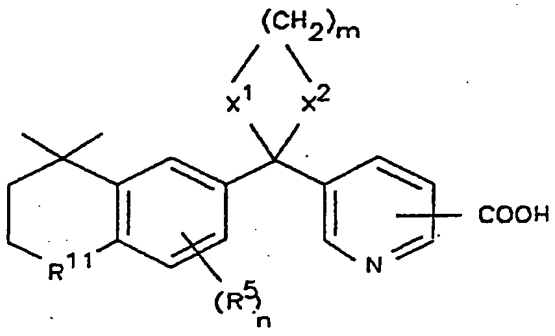
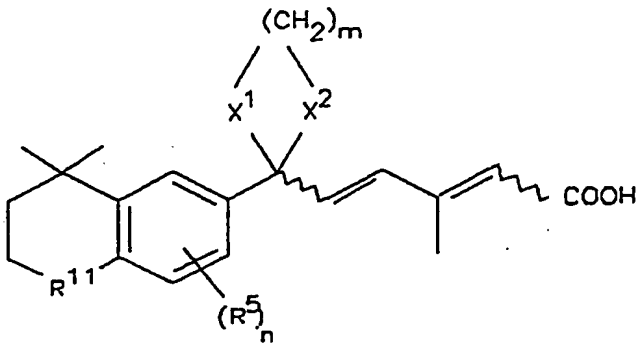


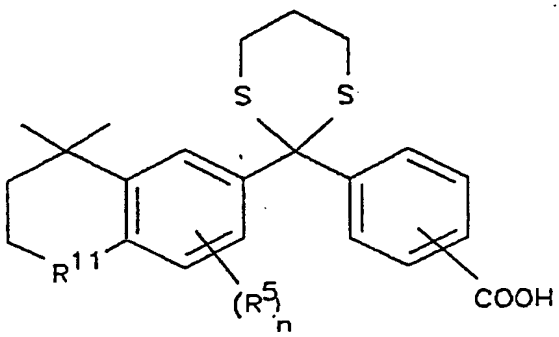
Examples of preferred structures encompassed by formula (II) thus include

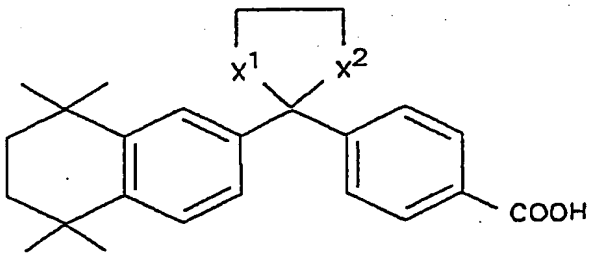












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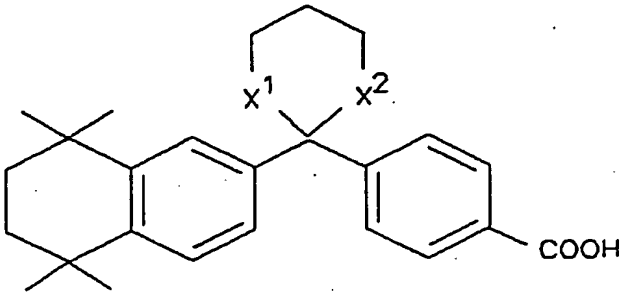
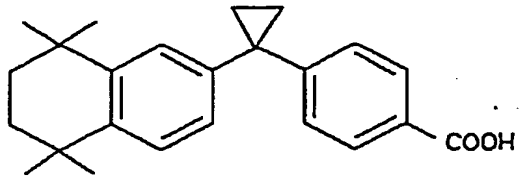
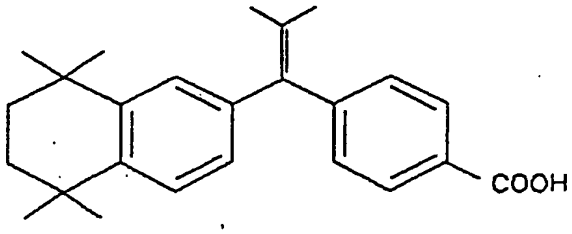
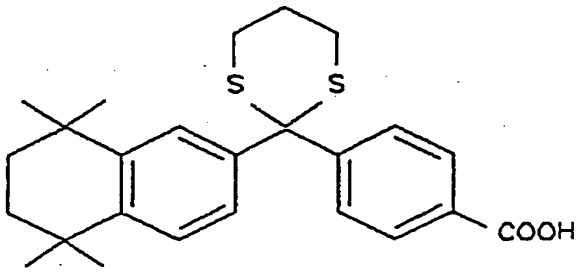
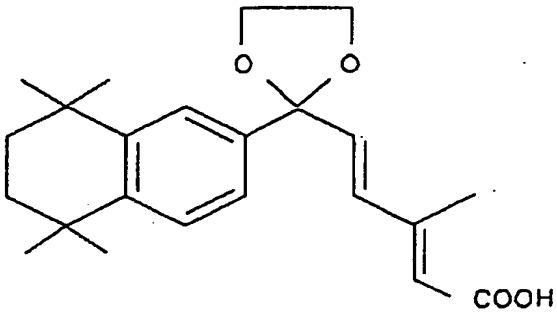


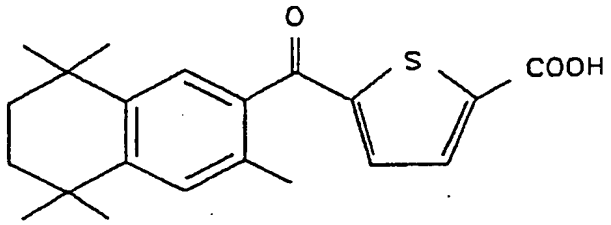
Examples of specific and particularly preferred compounds within the class of compounds defined by formula (II) include:

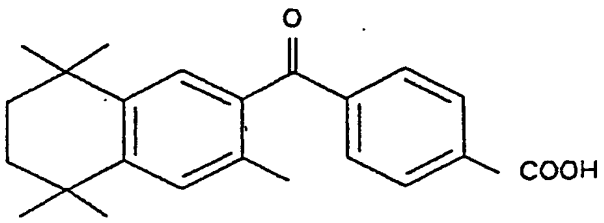


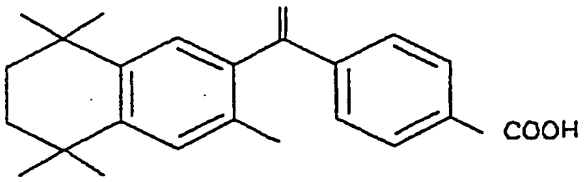




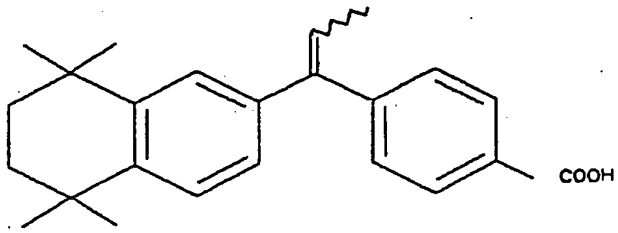








and



The invention also encompasses pharmaceutically acceptable nontoxic ester, amide and salt derivatives of those compounds of formula (I) containing a carboxylic acid moiety.

Pharmaceutically acceptable salts are prepared by treating the free acid with an appropriate amount of pharmaceutically acceptable base. Representative pharmaceutically acceptable bases are ammonium hydroxide, sodium hydroxide, potassium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, ferrous hydroxide, zinc hydroxide, copper hydroxide, aluminum hydroxide, ferric hydroxide, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, lysine, arginine, histidine, and the like. The reaction is conducted in water, alone or in combination with an inert, water-miscible organic solvent, at a temperature of from about 0°C to about 100°C, preferably at room temperature. The molar ratio of compounds of structural formula (I) to base used are chosen to provide the ratio desired for any particular salts. For preparing, for example, the ammonium salts of the free acid starting material--a particular preferred embodiment herein--the starting material can be treated with approximately one equivalent of pharmaceutically acceptable base to yield a neutral salt. When calcium salts are prepared, approximately one-half a molar equivalent of base is used to yield a neutral salt, while for aluminum salts, approximately one-third a molar equivalent of base will be used.

Ester derivatives are typically prepared as precursors to the acid form of the compounds--as illustrated in the Examples below--and accordingly may serve as prodrugs. Generally, these derivatives will be lower alkyl esters such as acetate, propionate, and the like. Amide derivatives $-(CO)NH_2$, $-(CO)NHR$ and $-(CO)NR_2$, where R is lower alkyl, may be prepared by reaction of the carboxylic acid-containing compound with ammonia or a substituted amine (as illustrated in Example VII below).

Synthetic Methods:

The compounds of the invention may be readily synthesized using techniques generally known to synthetic organic chemists. Suitable experimental methods for making and derivatizing bridged bicyclic aromatic compounds are described, for example, in the Maignan et al. patents summarized above, the disclosures of which patents are hereby incorporated by reference. Methods for making specific and preferred compounds of the present invention are described in detail in Examples III - XVII below.

Utility and Administration:

The compounds of the invention defined by structural formula (I), including the pharmacologically acceptable esters, amides or salts thereof, are useful to elicit and/or regulate selective gene expression by receptors in the retinoic acid family and to control cell differentiation processes regulated by retinoids, vitamin D and/or thyroid hormone. As noted above, the compounds of the invention are thus useful for treating acne, leukemia, psoriasis, skin aging, bone calcification and energy levels, as well as other indications related to cellular processes regulated by retinoic acid, vitamin D, thyroid hormone and 9-cis-retinoic acid.

The compounds of the invention may be conveniently formulated into pharmaceutical compositions composed of one or more of the compounds in association with a pharmaceutically acceptable carrier. See, e.g., Remington's Pharmaceutical Sciences, latest edition, by E.W. Martin (Mack Publ. Co., Easton PA) discloses typical carriers and conventional methods of preparing pharmaceutical compositions that may be used in conjunction with the preparation of formulations of the inventive compounds.

The compounds may be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, topically, transdermally, or the like, although oral or topical administration is typically preferred. The amount of active compound administered will, of course, be dependent on the subject being treated, the subject's weight, the manner of administration and

the judgment of the prescribing physician. Generally, however, dosage will approximate that which is typical for the administration of retinoic acid, and will preferably be in the range of about 2 μg/kg/day to 2 mg/kg/day.

Depending on the intended mode of administration, the pharmaceutical compositions may be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, suspensions, lotions, creams, gels, or the like, preferably in unit dosage form suitable for single administration of a precise dosage. The compositions will include, as noted above, an effective amount of the selected drug in combination with a pharmaceutically acceptable carrier and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, etc.

For solid compositions, conventional nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc, cellulose, glucose, sucrose, magnesium carbonate, and the like. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc., an active compound as described herein and optional pharmaceutical adjuvants in an excipient, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, referenced above.

For oral administration, fine powders or granules may contain diluting, dispersing, and/or surface active agents, and may be presented in water or in a syrup, in capsules or sachets in the dry

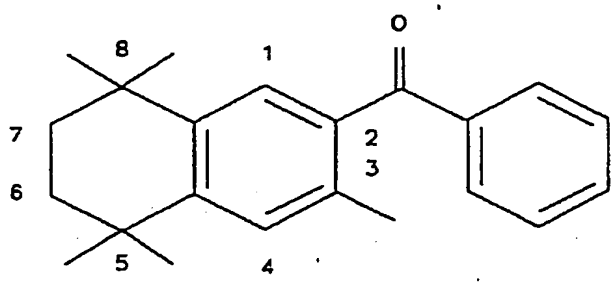
state, or in a nonaqueous solution or suspension wherein suspending agents may be included, in tablets wherein binders and lubricants may be included, or in a suspension in water or a syrup. Where desirable or necessary, flavoring, preserving, suspending, thickening, or emulsifying agents may be included. Tablets and granules are preferred oral administration forms, and these may be coated.

Parenteral administration, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system, such that a constant level of dosage is maintained. See, e.g., U.S. Patent No. 3,710,795, which is incorporated by reference herein.

Experimental

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the methods, compositions, and compounds claimed herein are made and evaluated, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C and pressure is at or near atmospheric.

In describing the location of groups and substituents, the following numbering system will be employed throughout the examples:



EXAMPLE I

Identification of homodimer formation

9-cis-RA induces RXR homodimer binding on the TREpal

Although RXRs have been shown to bind RA response elements (RAREs) when used at high concentrations,[28,30,31] more recent investigations revealed that RXR exists mainly as monomers in solution[10] and that effective DNA interaction requires heterodimer formation with RARs or TRs or VDR[15,16,26-29]. Binding of the heterodimers

to a variety of response elements was found to be ligand independent[32]. The newly discovered natural RA isomer, 9-cis-RA, has been reported to be an effective activator of RXRs in Drosophila Schneider cells that are known to contain neither RAR nor TRs[17,24]. If 9-cis-RA is indeed a true ligand for RXRs, one might expect that this ligand modulates RXR response element interaction. We therefore investigated the effect of 9-cis-RA on RXRα binding to the palindromic TRE (TREpal), an RXR responsive element[14], in the absence and presence of coreceptors (TRs and RARs).

The cloning of the receptor cDNAs for RXRα, RARß, or TRα into the pBluescript (Stratagene, San Diego, CA) have been described previously [26]. Flag-RXRα was constructed as described previously[33] by ligation of a double-stranded oligonucleotide containing an ATG codon and a DNA sequence encoding Flag (Arg-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) [SEQ ID NO:1] to the N-terminus of RXRα. The fusion product was then cloned into pBluescript. The synthesis of receptor proteins using in vitro transcription/translation system and gel retardation assays using synthesized receptor proteins and the double-stranded TREpal[34] were as described[33]. 9-cis-RA (m.p. 184-187°C) was prepared from 9-cis-retinal by a two-step sequence of MnO_2 oxidation in the presence of HOAc-MeOH[35] to give the methyl ester (69%) followed by hydrolysis (80%) in 0.5 N KOH in 25% aq. MeOH and crystallization (MeOH); HPLC (Novapak C_{18}, 32% MeCN, 27% MeCH, 16% I-PrOH, 24% H_2O, 1% HOAc, 1.9 mL/min, 260nM) t_R 15.4 min (100%). To analyze the effect of hormones, the receptor proteins were incubated with appropriate concentrations of hormone at room temperature for 30 min before performing the DNA binding assay. When anti-Flag antibody (Immunex, Seattle, WA) was used, 1 μl of the antiserum was incubated with receptor protein for another 30 min at room temperature before performing the DNA binding assay.

While in the absence of ligand, RXR alone did not bind effectively to the TREpal and required TR or RAR for response element interaction, binding of RXR was dramatically increased in the presence of 9-cis-RA (Fig. 1a) and did not require TRs or RARs. The RXR-specific band observed in the presence of 9-cis-RA was as prominent as

the bands obtained with the heterodimeric TR-RXR and RAR-RXR complexes. The RXR complex migrated more slowly than the TR-RXR complex at a position very similar to that of the RAR-RXR heterodimer-TREpal complex. These data therefore demonstrate that 9-cis-RA induces RXR homodimer formation. Due to migration of the RAR-RXR complex at the same position as the 9-cis-RA induced RXR homodimer complex, we were unable to determine in this experiment whether both complexes were formed. Remarkably, all-trans-RA when added at a concentration of 10^{-6} M, also induced to some degree RXRα homodimer binding whereas T_3 did not. Although the RA effect could be observed with several freshly prepared RA solutions, it is not clear at this point whether this homodimer formation in the presence of RA is due to 9-cis-RA impurities in the all-trans-RA solutions used here, or is a direct effect of all-trans-RA (see below). Interestingly, although it was reported that 9-cis-RA is capable of binding to RAR[24], unlike its effect on RXR, 9-cis-RA was not found to induce RAR homodimer binding to the TREpal.

To provide further evidence that the observed complex in the presence of 9-cis-RA indeed was an RXR complex, we performed the DNA binding experiment with Flag-RXR, a derivative that carries an 8 amino acid aminoterminal epitope recognized specifically by anti-Flag (αF) antibody[33]. As shown in Fig. 1b, αF supershifted the 9-cis-RA induced complexes, while a nonspecific antibody did not. This proves that the complex observed with the TREpal in the presence of 9-cis-RA indeed contained RXR protein. To examine how dependent the 9-cis-RA induced homodimer formation is on the concentration of RXR protein, increasing concentrations of in vitro translated RXR protein were mixed with the labeled TREpal in the presence or absence of 9-cis-RA (Fig. 2a). When these mixtures were analyzed by the gel retardation assay, a strong cooperative effect in homodimeric DNA binding was seen, positively dependent on the RXRα protein concentration (Fig. 2a,b). Although slight binding of RXR can be observed when high concentrations of RXR were used, 9-cis-RA was required for efficient complex formation at all receptor concentrations used. We further determined the concentrations of 9-cis-RA required for homodimer complex formation at all receptor concentrations used. We further

determined the concentrations of 9-cis-RA required for homodimer complex formation and observed a significant effect already at 10^{-9} M while optimal binding was seen at 10^{-8} M (Fig. 2c). Thus, effective RXR homodimer DNA interaction is dependent on RXR protein concentration and can occur at low levels of 9-cis-RA.

9-cis-RA induced homodimeric interaction with specific response elements

RXR containing heterodimers have a highly specific interaction with various natural response elements in that TR-RXR heterodimers only bind strongly to TREs but not to RAREs, whereas the opposite is true for RAR-RXR heterodimers[15,32]. We examined the sequence requirement of DNA binding of 9-cis-RA induced RXR homodimer using a number of natural and synthetic response elements (Fig. 3a).

Gel retardation assays using in vitro synthesized receptor protein are described in the Fig. 1 legend. The following oligonucleotides and their complements were used as probes in Figs. 3 and 4. ApoAI-RARE, a direct repeat response element with 2 bp spacer[31], gatcAGGGCAGGGGTCAAGGGTTCAGTgatc [SEQ ID NO:2]; CRBPII-RARE, a direct repeat RXR-specific response element with 1 bp spacer[30], gatcCAGGTCACAGGTCACAGGTCACAGTTCAAgatc [SEQ ID NO:3]; βRARE, a direct repeat of RA response element present in RARβ promoter[36,37], gatctGTAGGGTTCACCGAAAGTTCACTCagatc [SEQ ID NO:4]; CRBPI-RARE, a direct repeat RA specific response element present in rat CRBPI promoter[38], gatccAGGTCAAAAAGTCAGgatc [SEQ ID NO:5]; MHC-TRE, a direct repeat T_3 specific response element present in rat α-myosin heavy chain gene[40], gatcCTGGAGGTGACAGGAGGACAGCgatc [SEQ ID NO:6]; ME-TRE, a direct repeat T_3 specific response element present in the rat malic enzyme gene[41], gatcCAGGACGTTGGGGTTAGGGGAGGACAGTGGgatc [SEQ ID NO:7]; DR-4, an idealized direct repeat T_3 specific response element with 4 bp spacer[39], gatcTCAGGTCATCCTCAGGTCAgatc [SEQ ID NO:8]; DR-5, an idealized direct repeat RA specific response element with 5 bp spacer[39], gatcTCAGGTCATCCTCAGGTCAgatc [SEQ ID NO:9]; ERE, a perfect palindromic ER response element[42], gatcTCAGGTCACTGTGACCTGAgatc [SEQ ID NO:10]. The sequence of TREpal [SEQ ID NO:11] is shown for comparison.

ApoAI-RARE (a direct repeat response element that contains a 2 bp spacer), which has been suggested to be RXR-specific[31], resulted in a strong RXR complex in the presence of 9-cis-RA and to a lesser degree with RA (10^{-6} M). The RXR-RAR heterodimer also bound effectively to this response element. Since the heterodimer complex migrated at the same position as RXR homodimers, the effect of 9-cis-RA on RXR-RAR heterodimers cannot be clearly determined. RAR homodimer binding was not induced by 9-cis-RA (Fig. 3b). When we investigated 9-cis-RA induced RXR binding to another RXR responsive element[30], the CRBPII-RARE, we observed a complex that migrated more slowly than the heterodimer (in the absence of ligand), while in the presence of 9-cis-RA and RAR, both homodimer and heterodimer binding appeared to be reduced in their intensity (Fig. 3); a similar effect was seen at high RA concentrations or when both 9-cis-RA and RA were present. 9-cis-RA also induced RXR interaction with the βRARE (Fig. 4a), the RAR response element from the human RARβ promoter[36,37] that contains a 5 bp spacer. However, the RXR homodimer band was considerably weaker than the RAR-RXR heterodimer band at the protein concentrations used. Interestingly, another natural RARE derived from the rat CRBPI promoter[38], that like the ApoAI-RARE contains a 2 bp spacer, did not show any binding of RXR in the presence of 9-cis-RA (Fig. 4a), indicating that the actual sequence of the repeated core motif is critical for RXR homodimer binding. Similarly, the DR-5-RARE, a perfect repeat element derived from the β-RARE[39] did not exhibit interaction with RXR in the presence of 9-cis-RA, while it interacted strongly with RXR when RARE was present (Fig. 4a).

To examine whether RXR homodimer binding is specific to certain RAREs, we also performed gel shift experiments with the T_3 response elements from the rat α-myosin heavy chain promoter (MHC-TRE)[40], the rat malic enzyme (ME-TRE)[41] and the perfect repeat DR-4[39]. In all three cases, specific binding of RXR in the presence or absence of 9-cis-RA could not be observed, while all three response elements bound effectively TR/RXR heterodimers (Fig. 4b), consistent with the notion that these elements are not induced by retinoids. Similarly, the perfect palindromic ERE[42] also did not interact with RXR homodimers (Fig. 4c).

9-cis-RA induced homodimer formation occurs in the absence of DNA

It was reported the RXR exists mainly as monomer in solution[16]. An important question is whether 9-cis-RA induced RXR homodimers, like the RXR containing heterodimers, can form in solution in the absence of DNA. To address this question we took advantage of the Flag-RXR derivative that can be specifically precipitated with anti-Flag antibody while RXR wild type cannot.

Flag-RXRα was cloned in frame in the expression vector pGex 2T (Pharmacia) and was expressed in bacteria using the procedure provided by the manufacturer. Protein was partially purified on a prepacked glutathione sepharose 4B column (Pharmacia) and tested for its function by gel retardation assays and western blotting using anti-Flag antibody. Immunocoprecipitation assay was performed essentially as described[26]. Briefly, 10 μl of ^{35}S-labeled in vitro synthesized RXRα protein was incubated with 5 μl (approximately 0.1 μg) of partially purified bacterially expressed Flag-RXRα fusion protein or similarly prepared glutathione transferase control protein in 100 μl buffer containing 10^{-7} M 9-cis-RA, 50 mM KCl and 10% glycerol for 30 min at room temperature. When assayed in the presence of chemical cross-linker or oligonucleotides, we added 2 μl of 100 mM dithiobis succinimidylpropionate (DSP) dissolved in DMSO or 10 ng of oligonucleotide and continued the incubation at room temperature for 15 min. The reaction mixtures were then incubated with 1 μl of anti-Flag antibody or nonspecific preimmune serum for 2 h on ice. Immune complexes were precipitated by adding 50 μl of protein-A-sepharose slurry and mixing continuously in the cold room for 1 h. The immune complexes were washed extensively with cold NET-N buffer (20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM DTT, 0.5% NP-40) containing 10^{-7} M 9-cis-RA, boiled in SDS sample buffer and resolved by SDS-polyacrylamide gel electrophoresis. The gel was fixed, dried and visualized by autoradiography.

When we mixed Flag-RXR with in vitro labeled ^{35}S-RXR protein, the labeled RXR could be coprecipitated in the presence of anti-Flag antibody but not in the presence of nonspecific serum (Fig.

5). Coprecipitation efficiency was slightly increased in the presence of the ApoAI-RARE but not in the presence of the MHC-TRE. In addition, incubation with a crosslinker (DSP) further enhanced coprecipitation of the labeled RXR. In all cases, specific coprecipitation was only observed in the presence of 9-cis-RA. These data, therefore, give strong support to the assumption that 9-cis-RA induced RXR homodimer formation occurs in solution and does not require RXR-DNA interaction.

Response element specific transcriptional activation by 9-cis-RA and RXRα

To investigate whether 9-cis-RA/RXRα homodimer response element interaction can be correlated with transcriptional activation of such response elements by the 9-cis-RA/RXR complex, we carried out a series of transient transfection assays in CV-1 cells, where we cotransfected receptor expression vectors with CAT reporter constructs that carried various response elements upstream of the tk promoter. CV-1 cells were transiently transfected using a modified calcium phosphate precipitation procedure as described previously[43]. CAT activity was normalized for transfection efficiency by measuring the enzymatic activity derived from the cotransfected ß-galactosidase expression plasmid (pCH110, Pharmacia). The transfected cells were grown in the absence or presence of 10^{-7} M 9-cis-RA or all-trans-RA.

With the TREpal containing reporter, we observed strong activation by RXRα in the presence of 9-cis-RA and little activation when RA was added. Activation could be further enhanced by cotransfection of RARα. In this case, however, RA also functioned as an effective activator although not as efficiently as 9-cis-RA. Overall, activation by 9-cis-RA in the presence of RARα and RXRα was approximately twice as strong as seen with RXRα alone, consistent with the DNA binding data where binding was observed by both the heterodimer and the homodimer in the presence of 9-cis-RA when both receptors were present (Fig. 6a). When we examined the βRARE (Fig. 6b), we observed that this response element was highly activated by endogenous CV-1 cell receptors consistent with previous observations[37,42], such that further activation by low concentrations of cotransfected receptors could not be observed. Interestingly,

however, 9-cis-RA was a more potent activator at 10^{-7} M than RA. These data thus indicate that CV-1 cells contain endogenous retinoid receptor activity that is particularly active on the βRARE and is responsive to 9-cis-RA.

The ApoAI element containing reporter was also very effectively activated by RXRα in the presence of 9-cis-RA (Fig. 6c) while RA did not induce above the level obtained in the absence of RXRα. Similar to the TREpal, maximal activation was seen when both receptors RXRα and RARα were cotransfected. Under these conditions RA also led to a strong activation. In contrast, the CRBPI element, where we did not observe DNA binding by RXR in the presence of 9-cis-RA, also was not activated by RXR and 9-cis-RA in the transient transfection studies (Fig. 6d) while RARα alone led to significant activation that was mostly 9-cis-RA dependent. The heterodimer RARαRXRα allowed maximal activation in the presence of 9-cis-RA. Not unexpectedly, no induction by RXRα and 9-cis-RA was observed on the MHC-TRE, the ME-TRE or the ERE. These in vivo analyses showed a very significant correlation to the results obtained with the in vitro DNA binding studies, in that strong activation by RXRα in the presence of 9-cis-RA is only observed on the response elements that strongly interact with the 9-cis-RA induced RXRα homodimer.

9-cis Retinoic acid inhibits activation by TR/RXR heterodimer

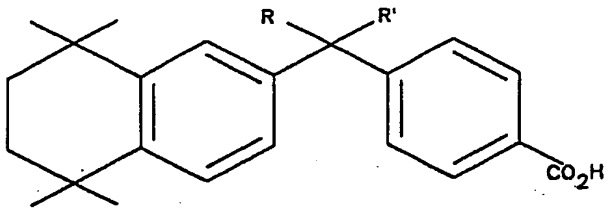
We have observed that a CAT reported gene that is activated by a thyroid hormone receptor/RXR heterodimer in the presence of thyroid hormone (T_3) can be inhibited by adding 9-cis-RA. This type of inhibition is most easily measured by using a transfection assay.

EXAMPLE II

Identification and selection of compounds which induce RXR activity

We used the TREpal-tk-reporter gene in a transient transfection assay essentially as described[51] to evaluate compounds for

induction of RXR activity. Briefly, CV-1 cells or Hep G2 cells were grown in DME medium supplemented with 10% fetal calf serum. Cells were plated at 1.0×10^5 per well in a 24-well plate 16-24 h before transfection. In general, 100 ng of reporter plasmid, 150 ng of ß-galactosidase expression vector (pCH110, Pharmacia), and variable amounts of receptor expression vector were mixed with carrier DNA (pBluescript) to 1,000 ng of total DNA per well. Chloramphenicol actyl transferase (CAT) activity was normalized for transfection efficiency by the corresponding ß-galactosidase activity as previously described[52]. As shown in Example I, the TREpal represents a response element that is activated by both RAR/RXR heterodimers and RXR homodimers. When the RXR expression vector is cotransfected with the TREpal-tk-reporter gene into CV-1 cells, all-trans-RA does not efficiently activate the reporter, whereas 9-cis-RA does. Evaluation of a series of retinoids indicated that several showed activity with RXR. The pharmacophoric elements of these structures were then combined and further modified to produce a subset of retinoids whose activation profiles for RXR were similar to that of 9-cis-RA. Induction curves for several retinoids active with RXR are shown in Fig. 7a. Interestingly, while none of the active compounds revealed activity at 10^{-8} M, all showed activities similar to 9-cis-RA at 10^{-7} M. We next used a reporter gene carrying the RARE[30] of the cytoplasmic retinol binding protein II (CRBPII), a response element that is only activated by RXR homodimers, but not by RAR/RXR heterodimers. The induction profiles obtained with this response element were similar to the TREpal responses (Fig. 7b). Thus, the synthetic retinoids SR11203, SR11217, SR11234, SR11235, SR11236, and SR11237 appeared to be effective activators of RXR*α*. The structures of the compounds are as follows:



SR11203	R, R' = $SCH_2CH_2CH_2S$	Ex. III	Cmp. 5
SR11217	R, R' = $(CH_3)_2C$	Ex. IX	Cmp. 20
SR11234	R, R' = SCH_2CH_2S	Ex. IV	Cmp. 7
SR11235	R, R' = OCH_2CH_2S	Ex. VI	Cmp. 12
SR11236	R, R' = $OCH_2CH_2CH_2O$	Ex. VII	Cmp. 14
SR11237	R, R' = OCH_2CH_2O	Ex. V	Cmp. 9

The activity rankings for this series of retinoids were the same for both the TREpal and CRBPII reporter genes. The ketal SR11237 was the most active, followed by the isopropylidenyl retinoid SR11217, the hemithioketal SR11235 and the thioketal SR11234. The dithiane SR11203 and dioxane SR11236 had the lowest activity. Conformational analysis indicated that these retinoids had spatial orientations of the lipophilic head and carboxyl terminus that were similar to those of 9-cis-RA and that activity could be related to the length and volume of the substituent group (CRR') linking the tetrahydronaphthalene and phenyl ring systems.

Given the showing in Example I that 9-cis-RA specifically activates RXRα by inducing RXRα homodimer formation, we investigated the retinoid-induced RXR homodimer binding to the TREpal using a gel retardation assay. Briefly, gel retardation assays were carried out essentially as described previously[33]. In vitro translated receptor receptor protein (1 to 5 ml depending on the translation efficiency) was incubated with the ^{32}P-labeled oligonucleotides in a 20-ml reaction mixture containing 10 mM Hepes buffer, pH 7.9, 50 mM KCl, 1 mM DTT, 2.5 mM MgCl, 10% glycerol, and 1 mg of poly(dI-dC) at 25°C for 20 minutes. The reaction mixture was then loaded on a 5% nondenaturing polyacrylamide gel containing 0.5 x TBE (1 x TBE = 0.089 M Tris-borate, 0.089 M boric acid, and 0.002 M EDTA).

In the absence of 9-cis-RA, RXR did not bind to this

response element. Retinoids SR11217, and SR11237 induced RXR homodimer binding to the response element in a concentration-dependent manner. Retinoid 11203, which behaved as a weak activator in the transient transfection assays, also induced only weak RXR binding. SR11231 which did not activate the RXR homodimer was also not able to induce RXR homodimer binding. Similar results were obtained with the CRBPII-RARE and the ApoAI-RARE. We have thus defined here a class of synthetic retinoids that activate RXRα by inducing homodimer formation and binding to DNA.

An important question was whether these RXR-active compounds, like 9-cis-RA, would also activate RAR/RXR heterodimers or whether they would be truly RXR selective. To analyze this, we used four different reporter constructs carrying either the i) rat cytoplasmic retinol binding protein I (CRBPI) gene RARE[38] that is only bound and activated by RAR/RXR heterodimers; ii) the RARβ2 gene promoter RARE[36,37], which is most effectively bound by heterodimers but also activated to some degree by RXR homodimers; iii) the CRBPII-RARE, which is activated only by RXR homodimers, and on which RAR represses RXR activity[30]; iv) the apolipoprotein AI (apoAI) gene RARE[31] that is bound and activated by RAR/RXR heterodimers as well as by RXR homodimers. The four different reporter constructs were cotransfected with RARα, RARβ, RXRα, or with RXRα and RARα together[51]. The retinoids were analyzed at a concentration of 5×10^{-7} M (a dose shown to yield almost full induction (Fig. 7)).

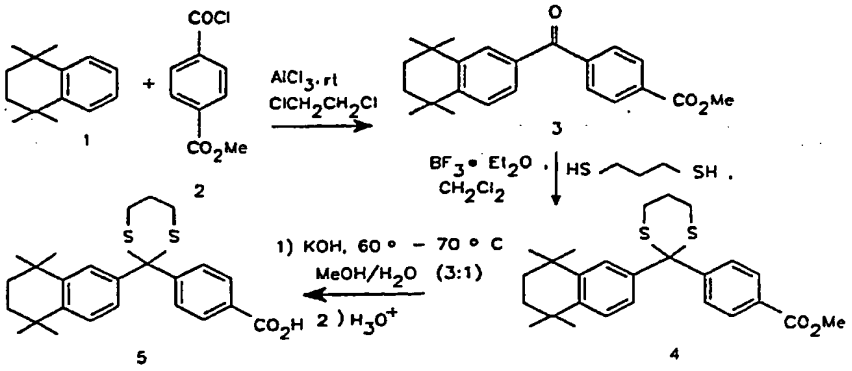
CV-1 cells were cotransfected with 100 ng reporter plasmid a) CRBPI-tk-CAT, b) βRARE-tk-CAT, c) CRBPII-tk-CAT, and d) apoAI-tk-CAT. Retinoids were applied at 5×10^{-7} M. Results of a representative experiment are shown.

The RXR-specific retinoids behaved strikingly different from 9-cis-RA (or RA) in that they only activated RXR homodimers but not RAR/RXR heterodimers. As with 9-cis-RA, both SR11217 and SR11237 were strong activators of the CRBPII-RARE (i.e. the RARE that is significantly activated only by the RXR homodimer). However, in contrast to 9-cis-RA, they did not induce the CRBPI-RARE that is

activated only by the RAR/RXR heterodimer. Thus, while SR11217 and SR11237 behaved very similarly to 9-cis-RA on the CRBPII-RARE, they showed no response on the CRBPI-RARE, where 9-cis-RA is the optimal activator. The βRARE was slightly activated by SR11217 and SR11237, consistent with the relatively low affinity of RXR homodimers for this response element. The apoAI-RARE was most effectively activated by RAR/RXR heterodimers in the presence of 9-cis-RA. In addition to the activity found in CV-1 cells, a significant and RXR-specific activation by retinoids SR11217 and SR11237 was also seen in various other cell lines, including Hep G2 cells, where a particular high response was seen. RARα and RARβ, when cotransfected alone, were not activated significantly by any of the synthetic retinoids on any of the response elements tested. Similar negative results were obtained for RARγ. RARα and β are assumed to form heterodimers with endogenous RXR-like proteins in CV-1 cells, thus these heterodimers are also unresponsive to the synthetic retinoids.

Our data demonstrate that we have identified a novel class of retinoids that specifically induces RXR homodimer formation and that activates RXR homodimers on specific response elements but not RAR/RXR heterodimers. These retinoids allow the specific activation of RXR-selective response pathways, while not inducing RAR-dependent response pathways. These retinoids provide a much more restricted physiological response than RA isomers or other retinoids presently used.

Example III



(a.) Methyl 4-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carbonyl]benzoate (3):

To a suspension of aluminum chloride (1.13 g, 8.5 mmol) in 1.5 mL of 1,2-dichloroethane at 0°C under argon was added a solution of 1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene 1 (1.45 g, 7.7 mmol) (Kagechika, H., et al., J. Med. Chem. 31:2182 (1988)) and 4-carbomethoxybenzoyl chloride 2 (1.56 g, 7.9 mmol) (4-carbomethoxybenzoyl chloride 2 was obtained from mono-methyl terephthalate, which is readily available from Aldrich, in one step ($SOCl_2$, DMF)) in 6 mL of 1,2-dichloroethane. The resulting solution was brought to room temperature and stirred thereafter for 16 h. The reaction mixture was poured onto ice water and extracted with 40% ethyl acetate/hexane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine. The solution was dried over anhydrous $MgSO_4$, filtered and concentrated to afford an orange solid (4.5 g). Flash chromatography (50% dichloromethane/hexane) yielded the desired product 3 as a pale yellow solid (2.07 g). Recrystallization from dichloromethane/hexane afforded the desired product 3 as a white, crystalline solid (1.96 g, 50%): m.p. 146-148°C; R_f 0.14 (50% CH_2Cl_2/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(b.) [2(5,6,7,8-Tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-2-(4-carbomethoxyphenyl)]-1,3-dithiane (4):

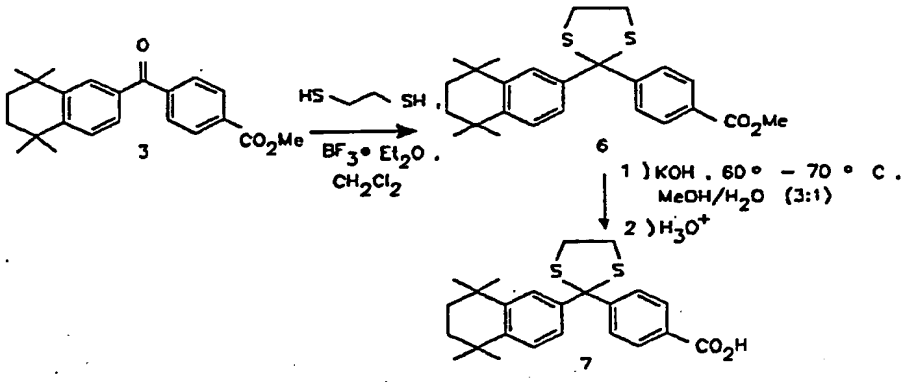
To a solution of the keto-ester 3 (97 mg, 0.277 mmol) in 3 mL of chloroform at 0°C under argon was added a solution of 1,3-propanedithiol (33 μL, 36 mg, 0.332 mmol) followed by boron trifluoride etherate (17 μL, 0.140 mmol). The resulting mixture was stirred at 0°C for 1 h and then warmed to room temperature overnight. The reaction mixture was quenched by pouring into saturated aqueous Na_2CO_3, and extracted with dichloromethane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to

afford a yellow solid (0.108 g). Recrystallization from ethyl acetate/hexane afforded the desired dithiane 4 as a white, crystalline solid (0.087 g, 71%): m.p. 195-197°C; R_f 0.32 (50% CH_2Cl_2/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(c.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-2-(4-carboxyphenyl)]-1,3-dithiane (5):

To a suspension of the ester 4 (85 mg, 0.193 mmol) in 75% aqueous methanol (2 mL) was added 0.024 g of potassium hydroxide and the mixture was stirred at 50°C for 2 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a white solid. Recrystallization from benzene/hexane afforded the desired acid 5 as a white, powder (0.076 g, 92%): m.p. 229-231°C. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

Example IV



(a.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carbomethoxyphenyl)]-1,3-dithiolane (6):

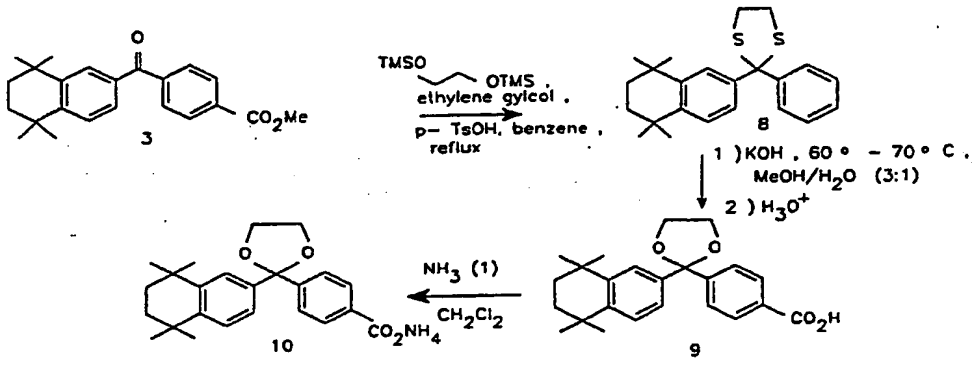
To a solution of the keto-ester 3 (80 mg, 0.228 mmol) in dichloromethane (2 mL) at 0°C under argon was added a solution of

ethanedithiol (26 mg, 0.27 mmol) in dichloromethane (0.5 mL) followed by boron trifluoride etherate (0.04 mL, 0.3 mmol). The resulting mixture was stirred at 0°C for 1 h and then warmed to room temperature overnight. The reaction mixture was quenched by pouring into saturated aqueous Na_2CO_3, and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a solid. Flash chromatography (30; 40% CH_2Cl_2/hexane) yielded the desired dithiane 6 as a white solid (0.088 g, 90%): m.p. 105-107°C; R_f 0.33 (50% CH_2Cl_2/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(b.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carboxyphenyl)]-1,3-dithiolane (7):

To a suspension of the ester 6 (85 mg, 0.199 mmol) in 75% aqueous methanol (3 mL) was added one pellet of potassium hydroxide (0.11 g) and the mixture was stirred at 70°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers when dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a white solid. Recrystallization from dichloromethane-hexane afforded the desired acid 7 as a white powder (0.064 g, 79%): m.p. 218-221°C. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

Example V



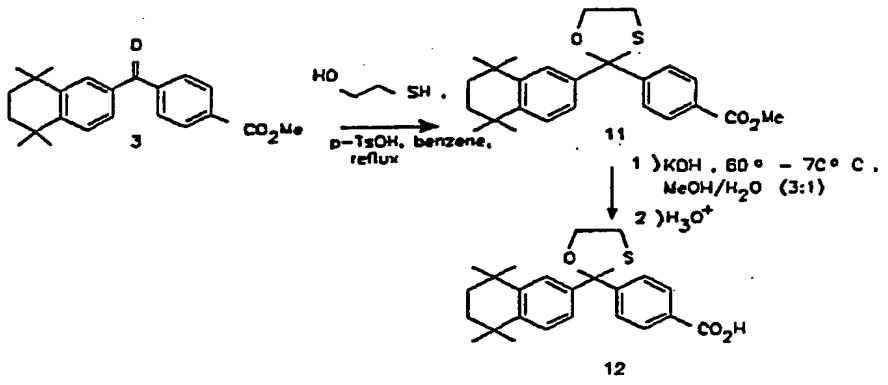
(a.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carbomethoxyphenyl)]-1,3-dioxolane (8):

To a solution of keto-ester 3 (80 mg, 0.228) in 1 mL of benzene was added ethylene glycol (1 mL), 1,2-bis(trimethylsilyloxy)ethane (2 mL) and a catalytic amount of p-TsOH. The reaction mixture was heated at reflux for 4 h and then cooled to room temperature. The solution was poured into saturated aqueous $NaHCO_3$ and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a solid. Flash chromatography (50% CH_2Cl_2/hexane) yielded the desired ketal 8 as a white solid (0.082 g, 91%): m.p. 145-147°C; R_f 0.16 (50% CH_2Cl_2/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(b.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carboxyphenyl)]-1,3-dioxolane ammonium salt (10):

To a suspension of the ester 8 (50 mg, 0.127 mmol) in 75% aqueous methanol (2 mL) was added one pellet of potassium hydroxide (0.1 g), and the reaction mixture was stirred at 70°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a white solid 9. The acid 9 was dissolved in dichloromethane (4 mL) under argon, and ammonia gas was condensed into the solution, which was stirred for 5 min at -33°C. The solution was warmed to room temperature for 20 min to evaporate the ammonia and concentrated to afford the ammonium salt 10 as a white powder (47 mg, 93%): m.p. 259-261°C. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

Example VI



(a.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carbomethoxyphenyl)]-1,3oxathiolane (11):

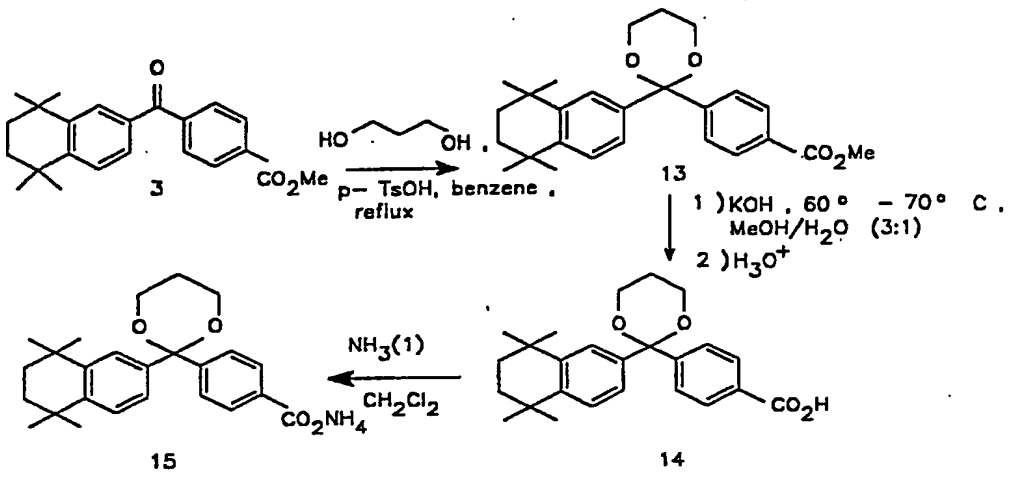
To a solution of keto-ester 3 (88 mg, 0.251) in 2 ml of benzene was added 2-mercaptoethanol (1 mL), and a catalytic amount of p-TsOH. The reaction mixture was heated at reflux overnight and then cooled to room temperature. The solution was poured into saturated aqueous $NaHCO_3$ and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a solid. Flash chromatography (50% CH_2Cl_2/hexane) yielded the desired ketal 11 as a white solid (0.09 g, 87%): m.p. 122-124°C; R_f 0.24 (50% CH_2Cl_2/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(b.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carboxyphenyl)]-1,3-oxathiolane (12):

To a suspension of the ester 11 (64 mg, 0.156 mmol) in 75% aqueous methanol (3 mL) was added one pellet of potassium hydroxide (0.12 g), and the reaction mixture was stirred at 75°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford

a white solid. Recrystallization from dichloromethane-hexane afford the desired acid 12 as a white powder (0.06 g, 97%): m.p. 216-217.5°C. The structure of the product was also confirmed using IR, ^{1}H NMR and mass spectroscopy.

Example VII



(a.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carbomethoxyphenyl)]-1,3-dioxane (13):

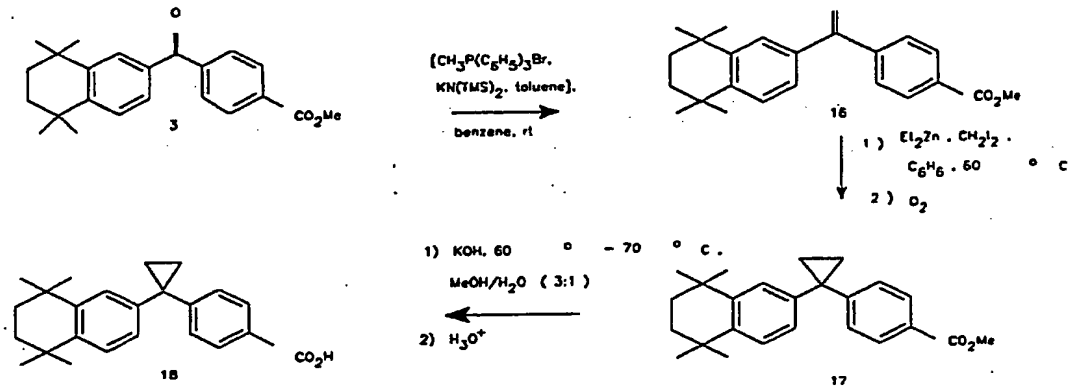
To a solution of keto-ester 3 (150 mg, 0.428 mmol) in 5 ml of benzene was added 1,3-propanediol (1.5 mL), and a catalytic amount of p-TsOH. The reaction mixture was heated at reflux overnight and then cooled to room temperature. The solution was poured into saturated aqueous $NaHCO_3$ and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a solid. Flash chromatography (50% CH_2Cl_2/hexane) yielded the desired ketal 13 as a white solid (0.164 g, 94%): m.p. 157-159°C; R_f 0.24 (5% ethyl acetate/hexane). The structure of the product was also confirmed using IR, ^{1}H NMR and mass spectroscopy.

(b.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carboxyphenyl)]-1,3-dioxane ammonium salt (15):

To a suspension of the ester 13 (0.1 g, 0.245 mmol) in

75% aqueous methanol (3 mL) was added one pellet of potassium hydroxide (0.12 g). The reaction mixture was stirred at 80°C for 30 min during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a white solid 14. The acid 14 was dissolved in dichloromethane (4 mL) under argon. Ammonia gas was condensed into the flask and the mixture was stirred for 5 min at -33°C. The solution was warmed to room temperature for 20 min to evaporate ammonia and concentrated to afford the ammonium salt 15 as a white powder (0.238 g, 97%): m.p. 228-230°C. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

Example VIII



(a.) Methyl 4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-ethenyl]benzoate (16):

To a suspension of methyltriphenylphosphonium bromide (0.78 g, 2.18 mmol) in 5 mL of benzene under argon at room temperature was added a 0.5 M solution of potassium hexamethyldisilazide in toluene (4.4 mL, 2.2 mmol), and the yellow solution was stirred for 5 min. A solution of keto-ester 3 (0.51 g, 1.455 mmol) in 7 mL of benzene was added, and the orange solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous

$NaHCO_3$ and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered through a plug of silica gel, and concentrated to afford a solid. Flash chromatography (30% dichloromethane/hexane) yielded the desired product 16 as a white solid (0.405 g, 80%): m.p. 117-118°C; R_f 0.2 (25% CH_2Cl_2/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

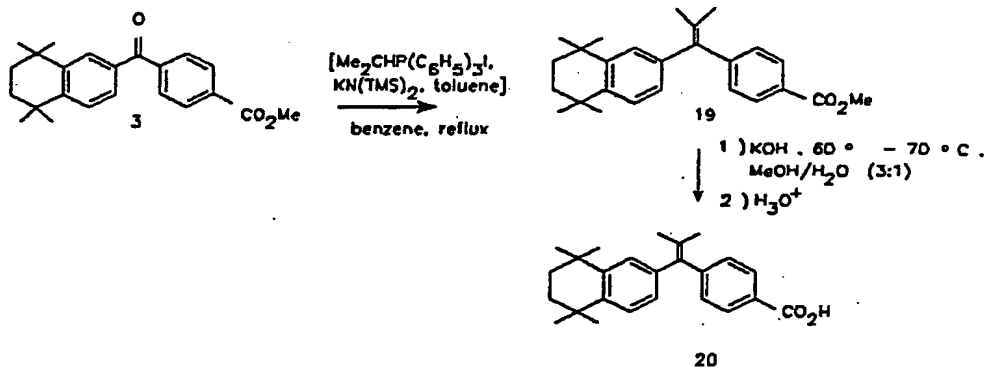
(b.) [1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-(4-carbomethoxyphenyl)]-cyclopropane (17):

To a solution of ethene 16 (0.130 g, 0.373 mmol) in 10 mL of benzene under argon at room temperature was added a 1 M solution of diethylzinc in hexane (5.6 mL, 5.6 mmol), and the reaction mixture was heated to 60°C. Diiodomethane (0.48 mL, 6.0 mmol) in 2 mL of benzene was added dropwise for 5 min. The reaction mixture was cooled to room temperature and oxygen was bubbled through for 3 h. The cloudy solution was diluted with 40% ethyl acetate/hexane and washed with aqueous hydrochloric acid, water and saturated aqueous $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a solid. Flash chromatography (30%; 40% CH_2Cl_2/hexane) yielded the desired product 17 as a white solid (0.08 g, 59%): m.p. 100-102°C; R_f 0.36 (50% CH_2Cl_2/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(c.) [1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-(4-carboxyphenyl)]cyclopropane (18):

To a suspension of the ester 17 (60 mg, 0.166 mmol) in 75% aqueous methanol (2 mL) was added one pellet of potassium hydroxide (0.12 g), and the reaction mixture was stirred at 70°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a white solid. Recrystallization from dichloromethane-hexane afforded the desired acid 18 as a white powder (0.055 g, 95%): m.p. 333-335°C. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

Example IX



(a.) Methyl 4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-methyl-1-propenyl]benzoate (19):

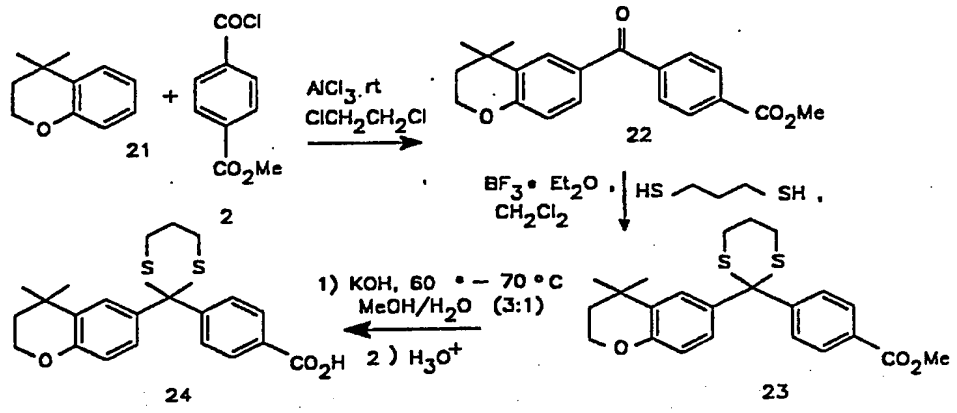
To a suspension of isopropyltriphenylphosphonium iodide (0.35 g, 0.807 mmol) in 3 mL of benzene under argon at room temperature was added a 0.5 M solution of potassium hexamethyldisilazide in toluene (1.8 mL, 0.89 mmol), and the red solution was stirred for 5 min. A solution of keto-ester 3 (0.169 g, 0.481 mmol) in 3 mL of benzene was added, and the red solution was heated to 110°C, while approximately 4 mL of benzene was distilled out. After 1 h, the reaction mixture was diluted with 40% ethyl acetate/hexane and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered through a plug of silica gel, and concentrated to afford a solid. Flash chromatography (40% dichloromethane/hexane) yielded the desired product 19 as a white powder (0.128 g, 71%): R_f 0.44 (50% CH_2Cl_2/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(b.) 4-[1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-methyl-1-propenyl]benzoic acid (20):

To a suspension of the ester 19 (0.115 g, 0.304 mmol) in 75% aqueous methanol (3 mL) was added one pellet of potassium hydroxide (0.12 g), The mixture was stirred at 75°C for 1 h during which time the material dissolved. The solution was cooled to room

temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford the desired acid 20 as a white powder (0.11 g, 99%): m.p. 204-206°C. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

Example X



(a.) Methyl 4-[(4,4-dimethyl-3,4-dihydro-2H-1-benzopyran-6-yl)carbonyl]benzoate (22) (Maignan, J., et al., BE 1,000,195):

To a suspension of aluminum chloride (1.6 g, 12 mmol) in 1 mL of 1,2-dichloroethane under argon at room temperature was added a solution of 4,4-dimethyl-3,4-dihydro-2H-1-benzopyran 21 (1.5 g, 9.25 mmol) (Dawson, M.I., et al., J. Med. Chem. 27:1516-1531 (1984)) and 4-carbomethoxybenzoyl chloride 2 (1.79 g, 9 mmol) in 9 mL of 1,2-dichloroethane. The reaction mixture was stirred overnight, poured onto ice-water and extracted with 40% ethyl acetate/hexane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine. The solution was dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a yellow solid (3.24 g). Flash chromatography (80% dichloromethane/hexane) yielded the desired product 22 as a white powder (1.42 g, 49%): m.p. 129-131°C; R_f 0.26 (CH_2Cl_2). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

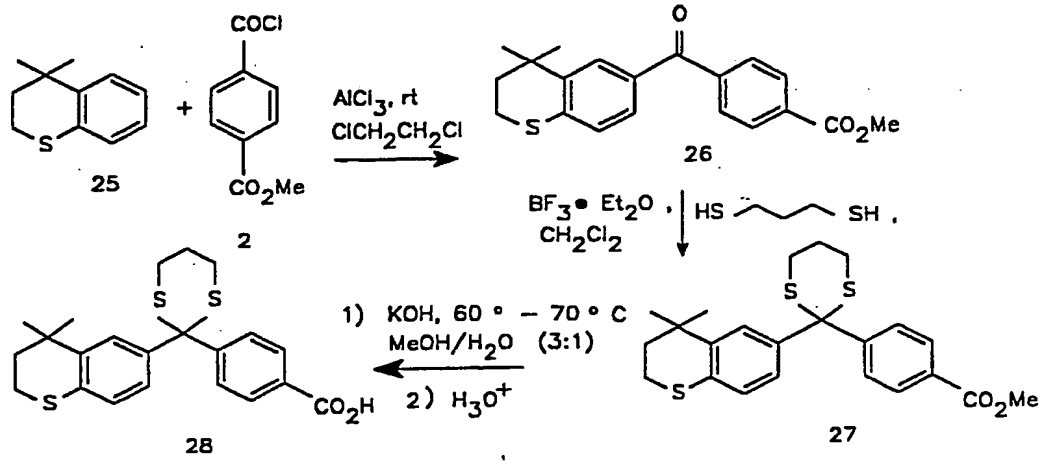
(b.) [2-(4,4-Dimethyl-3,4-dihydro-2H-1-benzopyran-6-yl)-2-(4-carbomethoxyphenyl)]-1,3-dithiane (23):

To a solution of the keto-ester 22 (0.152 g, 0.469 mmol) in dichloromethane (3 mL) at 0°C under argon was added 1,3-propanedithiol (0.061 g, 0.563 mmol), followed by boron trifluoride etherate (0.07 mL, 0.57 mmol). The resulting mixture was stirred at 0°C for 1 h and at ambient temperature overnight. The reaction mixture was quenched by pouring into saturated aqueous Na_2CO_3, and then extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford an oil. Flash chromatography (80% dichloromethane/hexane) yielded the desired dithiane 23 as a white solid (0.175 g, 90%): m.p. 103-105°C; R_f 0.2 (50% CH_2Cl_2/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(c.) [2-(4,4-Dimethyl-3,4-dihydro-2H-1-benzopyran-6-yl)-2-(4-carboxyphenyl)]-1,3-dithiane (24):

To a suspension of the dithiane 23 (0.145 g, 0.349 mmol) in 75% aqueous methanol (4 mL) was added one pellet of potassium hydroxide (0.106 g). The reaction mixture was stirred at 70°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a white solid. Recrystallization from dichloromethane-hexane afforded the desired acid 24 as a white powder (0.127 g, 90%): m.p. 204-205°C. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

Example XI



(a.) Methyl 4-[(4,4-dimethyl-3,4-dihydro-2H-1-benzothiopyran-6-yl)carbonyl]benzoate (26):

To a suspension of aluminum chloride (1.65 g, 9.25 mmol) in 1 mL of 1,2-dichloroethane under argon at room temperature was added a solution of 4,4-dimethyl-3,4-dihydro-2H-1-benzothiopyran 25 (1.65 g, 9.25 mmol) (Waugh, K.M., et al., J. Med. Chem. 28:116-124 (1985)) and 4-carbomethoxybenzoyl chloride 2 (1.8 g, 9.06 mmol) in 9 mL of 1,2-dichloroethane. The reaction mixture was stirred overnight and poured onto ice water and extracted with 40% ethyl acetate/hexane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine. The solution was dried over anhydrous $MgSO_4$, filtered and concentrated to afford a green solid (2.1 g). Flash chromatography (80% dichloromethane/hexane) yielded the desired product 26 as a white powder (1.23 g, 40%): m.p. 118-120°C; R_f 0.35 (CH_2Cl_2). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(b.) [2-(4,4-Dimethyl-3,4-dihydro-2H-1-benzothiopyran-6-yl)-2-(4-carbomethoxyphenyl)]-1,3-dithiane (27):

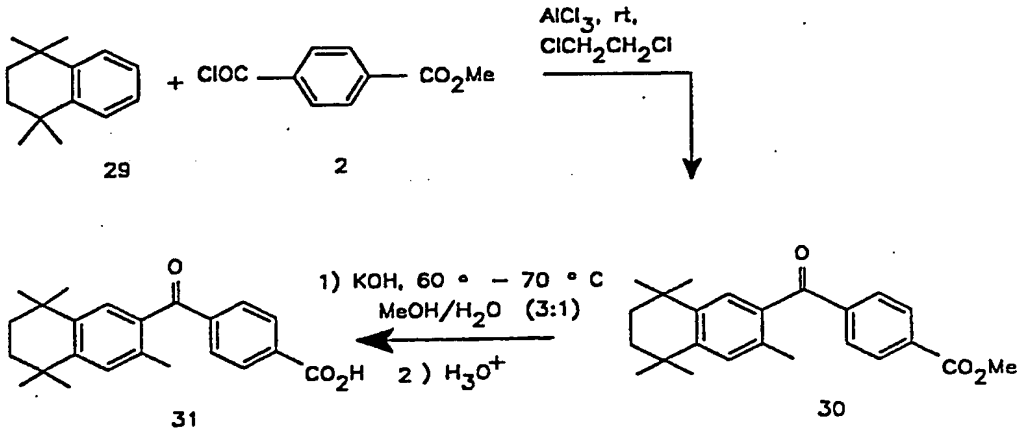
To a solution of the keto-ester 26 (0.12 g, 0.353 mmol) in dichloromethane (3 mL) at 0°C under argon was added 1,3-propandithiol (0.06 mL, 0.53 mmol) followed by boron trifluoride etherate (0.07 mL,

0.57 mmol). The resulting mixture was stirred at 0°C for 1 h and then warmed to room temperature overnight. The reaction mixture was quenched by pouring into saturated aqueous Na_2CO_3, and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford an oil. Flash chromatography (80% CH_2Cl_2/hexane) yielded the desired dithiane 27 as a white solid (0.145 g, 96%): m.p. 164-166°C; R_f 0.27 (50% CH_2Cl_2/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(c.) [2-(4,4-Dimethyl-3,4-dihydro-2H-1-benzothiopyran-6-yl)-2-(4-carboxyphenyl)]-1,3-dithiane (28):

To a suspension of the dithiane 27 (0.1 g, 0.232 mmol) in 75% aqueous methanol (4 mL) was added one pellet of potassium hydroxide (0.12 g). The reaction mixture was stirred at 75°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a white solid. Recrystallization from dichloromethane/hexane afforded the desired acid 28 as a white powder (0.089 g, 92%): m.p. 211-212.5°C. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

Example XII



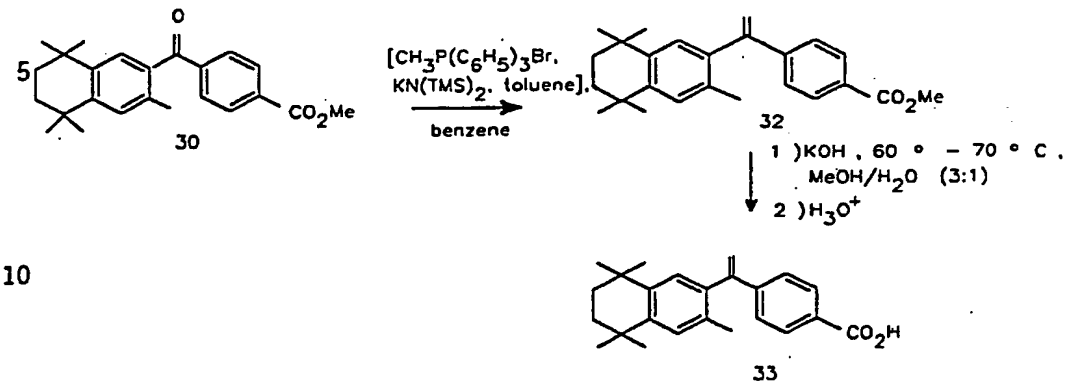
(a.) Methyl [4-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)carbonyl]benzoate (30):

To a suspension of aluminum chloride (1.10 g, 8.25 mmol) in 30 mL of 1,2-dichloroethane under argon at room temperature was added a solution of 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene 29 (1.52 g, 7.5 mmol) (Kagechika, H., et al., J. Med. Chem. 31:2182 (1988)) and 4-carbomethoxybenzoyl chloride 2 (1.57 g, 7.87 mmol) in 15 mL of 1,2-dichloroethane. The reaction mixture was stirred overnight and poured onto ice water and extracted with 40% ethyl acetate/hexane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine. The solution was dried over anhydrous $MgSO_4$, filtered and concentrated to afford a brown solid (2.56 g). Flash chromatography (60% dichloromethane/hexane) yielded the desired product 30 as a white, crystalline solid (1.733 g, 64 %): m.p. 146-149°C; R_f 0.11 (50% CH_2Cl_2/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(b.) [4-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)carbonyl]benzoic acid (31):

To a suspension of the ester 30 (0.120 g, 0.329 mmol) in 75% aqueous methanol (2 mL) was added potassium hydroxide (0.055 g). The reaction mixture was stirred at 60°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a white solid (0.109 g). Recrystallization from benzene/hexane afforded 31 as a white, crystalline solid (0.102 g, 89%): m.p. 209-212°C. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

Example XIII



(a.) Methyl 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)-1-ethenyl]benzoate (32):

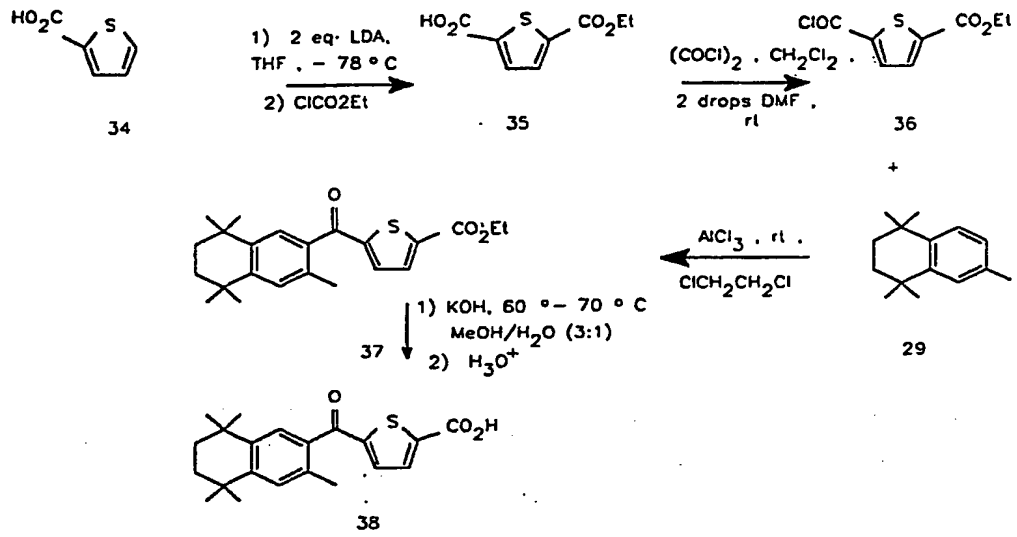
To a suspension of methyltriphenylphosphonium bromide (0.196 g, 0.55 mmol) in 1 mL of benzene under argon at room temperature was added a 0.5 M solution of potassium hexamethyldisilazide in toluene (1.2 mL, 0.6 mmol), and the yellow solution was stirred for 5 min. A solution of keto-ester 30 (0.1 g, 0.274 mmol) in 1.5 mL of benzene was added, and the orange solution was stirred for 3 h at room temperature. The reaction mixture was filtered through a plug of silica gel with 40% ethyl acetate/hexane. The filtrate was concentrated to afford a solid. Flash chromatography (30%; 40% dichloromethane/ hexane) yielded the desired product 32 as a white solid (0.077 g, 78%): m.p. 167-168°C; R_f 0.4 (50% dichloromethane/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(b.) [4-[1-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)-1-ethenyl]benzoic acid (33):

To a suspension of the ester 32 (0.058 g, 0.16 mmol) in 75% aqueous methanol (2 mL) was added one pellet of potassium hydroxide (0.1 g). The mixture was stirred at 70°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then

extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a white solid. Recrystallization from dichloromethane/hexane afforded the desired acid 33 as a white, crystalline solid (42 mg, 91%): m.p. 230-231°C. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

Example XIV



(a.) 5-Carboethoxythiophen-2-carboxylic acid (35):

To a solution of diisopropylamine (3.6 mL, 25.75 mmol) in 10 mL of THF at -78°C under argon was added a 1.6 M solution of n-butyllithium in hexane (16.1 mL, 25.75 mmol). The mixture was stirred for 15 min, and a solution of 2-thiophenecarboxylic acid 34 (1.5 g, 11.705 mmol) (2-thiophenecarboxylic acid 34 is readily available from Aldrich) in 5 mL of THF was added slowly. The mixture was stirred for 15 min, and ethyl chloroformate (2.7 mL, 28.33 mmol) was added. The mixture was stirred for 30 min at -78°C, and warmed to 0°C for another 30 min. The reaction mixture was poured into saturated aqueous $NaHCO_3$, and washed with 80% ethyl acetate/hexane. The aqueous layer was acidified with acetic acid and extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over

anhydrous $MgSO_4$, filtered, and concentrated to afford a yellow solid. Flash chromatography yielded the desired acid 35 (1.76 g, 75%) as a white solid: m.p. >300°C. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(b.) 5-Carboethoxythiophen-2-carbonyl chloride (36):

To a suspension of acid 35 (0.64 g, 3.2 mmol) in dichloromethane (20 mL) under argon at room temperature was added a 2.0 M solution of $(COCl)_2$ in dichloromethane (2.4 mL, 4.8 mmol) and two drops of DMF, and stirred overnight. Excess $(COCl)_2$ and dichloromethane were removed at reduced pressure, and the viscous, light, yellow product was dried overnight to afford the desired benzoyl chloride 36 as a yellow solid. The structure of the product was also confirmed using IR and ^1H NMR spectroscopy.

(c.) Ethyl 5-[(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)carbonyl]thiophen-2-carboxylate (37):

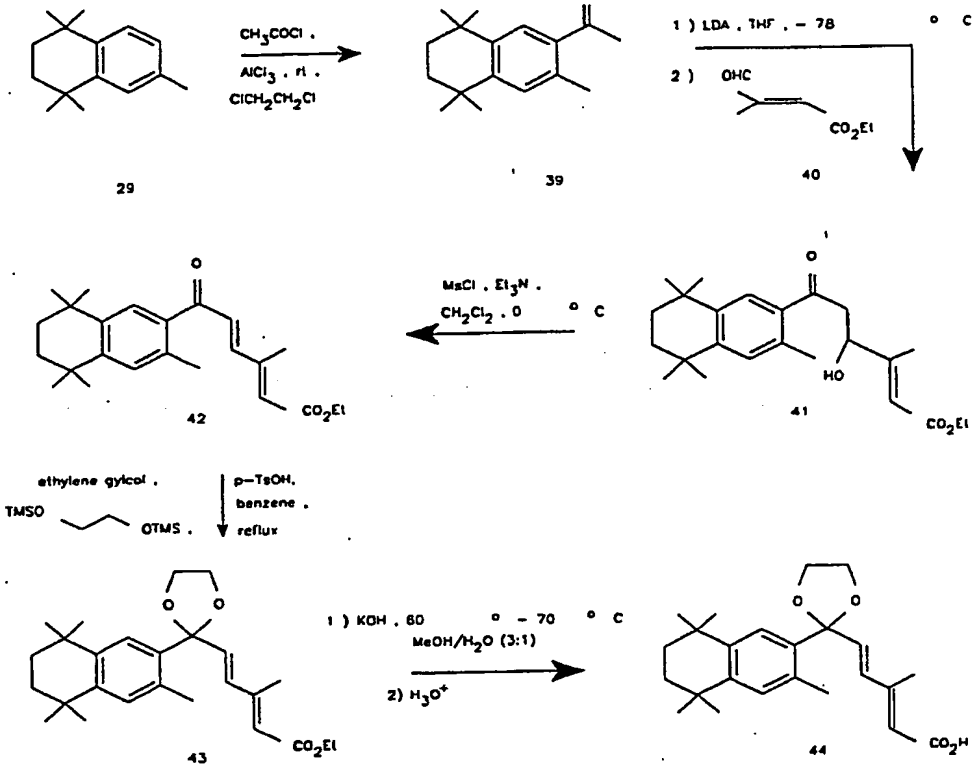
To a suspension of aluminum chloride (0.5 g, 3.75 mmol) in 1 mL of 1,2-dichloroethane under argon at room temperature was added a solution of 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene 29 (0.712 g, 3.52 mmol) and 36 (0.7 g, 3.2 mmol) in 3.5 mL of 1,2-dichloroethane dropwise. The reaction mixture was stirred overnight, and then poured onto ice water, and extracted with 40% ethyl acetate/hexane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine. The solution was dried over anhydrous $MgSO_4$, filtered and concentrated to afford a yellow solid (1.5 g). Flash chromatography (50% dichloromethane/hexane) yielded a light-yellow solid. Recrystallization from dichloromethane/hexane afforded 37 as a white crystalline solid (0.62 g, 50%): m.p. 111-112°C; R_f 0.2 (50% CH_2Cl_2/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(d.) 5-[(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)carbonyl]thiophen-2-carboxylic acid (38):

To a suspension of the ester 37 (50 mg, 0.13 mmol) in 75% aqueous methanol (3 mL) was added one pellet of potassium hydroxide (0.1 g). The reaction mixture was stirred at 70°C for 1.5 h during

which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a white solid (46 mg). Recrystallization from methanol afforded the desired acid 38 as a white crystalline solid (42 mg, 91%): m.p. 214-215°C. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

Example XV



(a.) 2-Acetyl-3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalene (39):

To a suspension of aluminum chloride (0.96 g, 7.17 mmol) in 1 mL of 1,2-dichloroethane under argon at room temperature was added a solution of 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethyl-naphthalene 29 (1.2 g, 5.93 mmol) and acetyl chloride (0.51 g, 6.52 mmol) in 9 mL of 1,2-dichloroethane. The reaction mixture was stirred

for 1 h and then was poured onto ice water and extracted with 40% ethyl acetate/hexane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine. The solution was dried over anhydrous $MgSO_4$, filtered through a plug of silica gel, and concentrated to afford a white solid 39 (1.45 g, 99%): m.p. 54-57°C; R_f 0.62 (10% ethyl acetate/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(b.) Ethyl (E)-4-hydroxy-3-methyl-6-oxo-6-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthalenyl)-2-hexenoate (41):

To a solution of diisopropylamine (0.5 mL, 3.52 mmol) in 7 mL of THF at -78°C under argon was added a 1.6 M solution of n-butyllithium in hexane (2.2 mL, 3.52 mmol). The mixture was stirred for 25 min, and a solution of ketone 39 (0.78 g, 3.2 mmol) in 4 mL of THF was added slowly. The mixture was stirred for 20 min, and a solution of ethyl (E)-3-formyl-2-butenoate 40 (0.45 g, 3.2 mmol) (ethyl (E)-3-formyl-2-butenoate 40 is readily available from Fluka) in 3 mL of THF was added slowly. The mixture was stirred for 35 min at -78°C, poured into saturated aqueous NH_4Cl, and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a light-yellow solid. Flash chromatography yielded the desired alcohol 41 as a white crystalline solid (0.98 g, 80%): m.p. 126-128°C; R_f 0.13 (10% ethyl acetate/hexane). The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(c.) Ethyl (2E,4E)-6-oxo-6-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2- naphthalenyl)-2,4-hexadienoate (42):

To a solution of 41 (0.33 g, 0.85 mmol) in 8 mL of THF at 0°C was added triethylamine (0.5 mL, 4 mmol), and a solution of methylsulfonyl chloride (0.106 g, 0.93 mmol) in 2 mL of THF slowly. The mixture was stirred for 1 h at 0°C and warmed to room temperature for 30 min. The mixture was filtered through a plug of silica gel with 10% ethyl acetate/hexane. The filtrate was concentrated to afford 42 as a light-yellow solid. Recrystallization from dichloromethane/hexane afforded the desired acid 42 as a yellow powder (0.3 g, 95%): m.p. 101-102°C; R_f 0.42 (10% ethyl acetate/hexane). The

structure of the product was also confirmed using IR, ^{1}H NMR and mass spectroscopy.

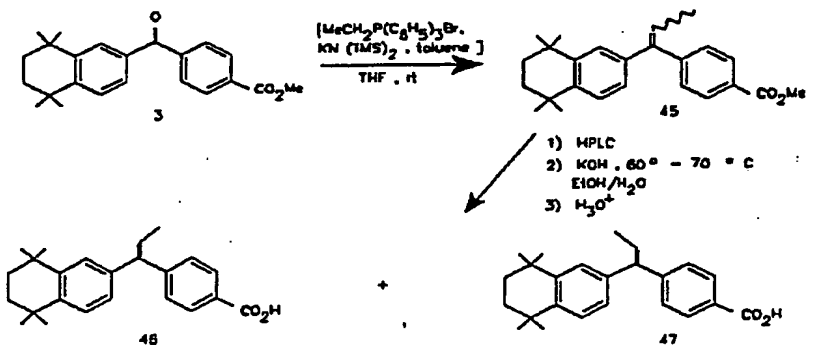
(d.) [2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)-2-(4-carboethoxy-2E,4E-3-methylbutadienyl)]-1,3-dioxolane (43):

To a solution of keto-ester 42 (0.12 g, 0.33) in 2 mL of benzene was added ethylene gylcol (0.8 mL), 1,2-bis(trimethylsilyloxy)ethane (2 mL) and a catalytic amount of p-TsOH. The reaction mixture was heated at reflux for 2 days and then cooled to room temperature. The solution was poured into saturated aqueous $NaHCO_3$ and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a solid. Flash chromatography (5% ethyl acetate/hexane) yielded the desired ketal 43 as a colorless oil (0.109 g, 80 %). : R_f 0.43 (10% ethyl acetate/hexane). The structure of the product was also confirmed using IR, ^{1}H NMR and mass spectroscopy.

(e.) [2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)-2-(4-carboxy-2E,4E-3-methylbutadienyl)]-1,3-dioxolane (44):

To a solution of the ester 43 (30 mg, 0.073 mmol) in 75% aqueous methanol (2 mL) was added one pellet of potassium hydroxide (0.1 g). The reaction mixture was stirred at 60°C for 0.5 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated to afford a white solid. Recrystallization from dichloromethane/hexane afforded the desired acid 44 as a white, crystalline solid (27 mg, 96 %): m.p. 189-190°C. The structure of the product was also confirmed using IR, ^{1}H NMR and mass spectroscopy.

Example XVI



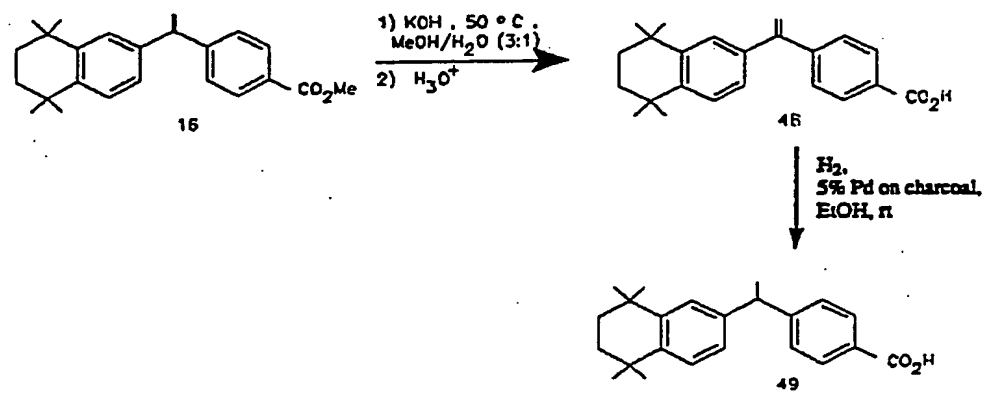
(E) and (Z)-4-[1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-1-yl]benzoic acid (46 and 47):

To a suspension of ethyltriphenylphosphonium bromide (160 mg, 0.43 mmol) in 1 mL of THF under argon at 0°C was added a 0.5 M solution of potassium hexamethyldisilazide in toluene (0.95 mL, 0.47 mmol), and the reaction mixture was stirred for 5 min. A solution of keto-ester 3 (100 mg, 0.28 mmol) in 0.8 mL of THF was added, and the orange solution was stirred at room temperature for 3 h. The reaction mixture was diluted with 20% ethyl acetate/hexane and filtered through a plug of silica gel with 20% ethyl acetate/hexane. The solution was concentrated to afford a yellow gum. Chromatography (38% dichloromethane/hexane) yielded the mixture of 45 as a pale-yellow gum (51 mg, 50%): R_f 0.43, 0.47 (40% CH_2Cl_2/hexane). Preparative HPLC (Waters Radialpak Novapak silica, 8 mm x 10 cm, 2% ether/hexane, 1.0 mL/min, 260 nm) gave a colorless gum 45a (20 mg, t_r = 9.8 min) and a white solid 45b (25 mg, t_r = 10.8 min).

To a suspension of the ester 45a (20 mg) in 0.5 mL of ethanol was added a 40% solution of aqueous potassium hydroxide (0.2 g), and the reaction mixture was stirred at 70 C under argon for 2 h during which time the material dissolved. The solution was then concentrated in an argon stream. The residue was cooled to room temperature, acidified with 1 N aqueous sulfuric acid to pH 2-3, and then filtered. The precipitate was repeatedly washed with water (6 x 1 mL) and dried to afford a white powder (20 mg). Recrystallization from ethyl acetate/hexane afforded the acid 46 as a white powder (16 mg, 16% overall yield): m.p. 212oC. The structure of the product was also confirmed using IR, ^{1}H NMR and mass spectroscopy. The regiochemistry of 46 was confirmed by ^{1}H nOe NMR spectroscopy.

The ester 45b was hydrolyzed as above to give 25 mg of pale-yellow solid. Recrystallization from ethyl acetate afforded the acid 47 as a pale-yellow powder (21 mg, 20% overall yield): m.p. 229oC. The structure of the product was also confirmed using IR, ^{1}H NMR and mass spectroscopy. The regiochemistry of 47 was confirmed by ^{1}H nOe NMR spectroscopy.

Example XVII



(a.) 4-[1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-ethenyl]benzoic acid (48):

To a suspension of the ester 16 (94 mg, 0.27 mmol) in 75% aqueous methanol (2 mL) was added potassium hydroxide (0.045 g), and the reaction mixture was stirred at 50°C for 14 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 2 N aqueous hydrochloric acid, and then extracted with ether. The combined organic layers were washed with water and brine. The organic solution was then dried over anhydrous $MgSO_4$, filtered and concentrated to afford a white solid. Recrystallization from benzene-hexane afforded the desired acid 48 as a white crystalline solid (0.074 g, 82%): m.p. 201-204°D. The structure of the product was also confirmed using IR, ^1H NMR and mass spectroscopy.

(b.) 4-[1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-ethyl]benzoic acid (49):

The olefin 48 (0.0105 g, 0.0314 mmol) was hydrogenated over 5% palladium on charcoal (1 mg) in 0.5 mL of ethanol at room temperature and atmospheric pressure. After one equivalent (0.7 mL) of hydrogen was taken up, the catalyst was removed by filtration

through a small Celite pad. The solvent was removed in vacuo to give the crude acid as a white solid (0.019 g). Recrystallization from benzene-hexane afforded the desired acid 49 as a white crystalline solid (0.0078 g, 74%): m.p. 186-188°C. The structure of the product was also confirmed using IR, ^{1}H NMR and mass spectroscopy.

The preceding examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may be alternatively employed.

Throughout this application various publications are referenced by numbers. Following is a complete citation to the publications. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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SEQUENCE LISTING

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(A) NAME: The Government of the United States of America, as represented by The Secretary
(B) STREET: 6011 Executive Blvd., Suite 325
(C) CITY: Rockville
(D) STATE: Maryland
(E) COUNTRY: United States of America
(F) POSTAL CODE (ZIP): 20852
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(I) TELEX: None

(ii) TITLE OF INVENTION: RXR HOMODIMER FORMATION

(iii) NUMBER OF SEQUENCES: 11

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: NOT YET ASSIGNED
(B) FILING DATE:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US/07/901/719
(B) FILING DATE: 16-JUN-1992

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Arg Tyr Lys Asp Asp Asp Asp Lys
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GATCAGGGCA GGGGTCAAGG GTTCAGTGAT C 31

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single.
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GATCCAGGTC ACAGGTCACA GGTCACAGTT CAAGATC 37

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GATCTGTAGG GTTCACCGAA AGTTCACTCA GATC 34

(2) INFORMATION FOR SEQ ID NO:5:

3 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATCCAGGTC AAAAAGTCAG GATC 24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GATCCTGGAG GTGACAGGAG GACAGCGATC 30

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GATCCAGGAC GTTGGGGTTA GGGGAGGACA GTGGGATC 38

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GATCTCAGGT CATCCTCAGG TCAGATC 27

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GATCTCAGGT CATCCTCAGG TCAGATC 27

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GATCTCAGGT CACTGTGACC TGAGATC 27

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GATCTCAGGT CATGACCTGA GATC 24

What is claimed is:

1. A method of screening a substance for the ability to affect the formation of a retinoid X receptor homodimer comprising combining the substance and a solution containing retinoid X receptors and determining the presence of homodimer formation.

2. The method of claim 1, wherein the presence of homodimer formation is determined by detecting the activation of transcription by the retinoid X receptor homodimer.

3. The method of claim 1, wherein the presence of homodimer formation is determined by coprecipitation.

4. The method of claim 1, wherein the effect is the induction of homodimer formation.

5. The method of claim 1, wherein the effect is the inhibition of homodimer formation.

6. The method of claim 1, wherein the retinoid X receptor is retinoid X receptor α.

7. The method of claim 4, further comprising determining if the substance induces a retinoid X receptor heterodimer and selecting the substance which selectively induces the formation of a retinoid X receptor homodimer.

8. A method of screening a substance for the ability to selectively induce the formation of a retinoid X receptor heterodimer over a retinoid X receptor homodimer comprising:

a) determining if the substance induces retinoid X receptor homodimer formation;

b) determining if the substance induces retinoid X receptor heterodimer formation; and

c) selecting the compound which selectively induces the formation of a retinoid X receptor heterodimer over a retinoid X receptor

homodimer.

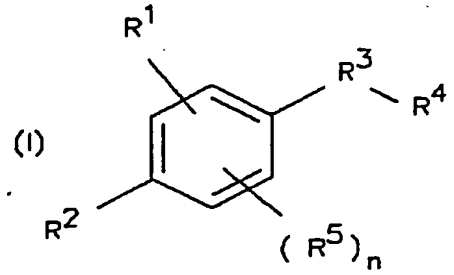
9. A method of screening a substance for an affect on a retinoid X receptor homodimer's ability to bind DNA comprising combining the substance with the homodimer and determining the affect of the compound on the homodimer's ability to bind DNA.

10. A method of screening a response element for binding with a retinoid X receptor homodimer comprising combining the response element with the retinoid X receptor homodimer and detecting the presence of binding.

11. The method of claim 10, wherein the presence of binding is detected by the transcriptional activation of a marker which is operably linked to the response element.

12. A purified retinoid X receptor homodimer.

13. A bicyclic aromatic compound having the structural formula



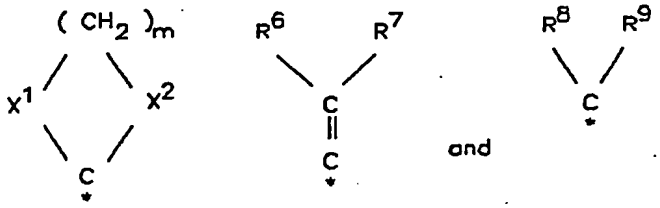
wherein:

R^1 is selected from the group consisting of lower alkyl and adamantyl;

R^2 is $-O-R^6$ or $-S-R^6$ where R^6 is lower alkyl; or

where R^1 is ortho to R^2, R^1 and R^2 may be linked together to form a 5- or 6-membered cycloalkylene ring, either unsubstituted or substituted with 1 to 4 lower alkyl groups, and optionally containing 1 or 2 heterocyclic atoms selected from the group consisting of O, S and NR where R is hydrogen or lower alkyl;

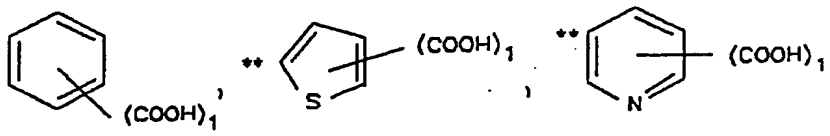
R^3 is selected from the group consisting of carbonyl,



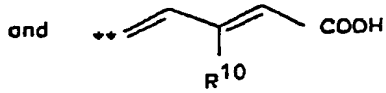
in which X^1 and X^2 are independently selected from the group consisting of O, S and methylene, wherein at least one of X^1 and X^2 is O or S, or wherein one of X^1 and X^2 is NR and the other is methylene, m is 2 or 3, R^6, R^7, R^8 and R^9 are independently hydrogen or lower alkyl, with the proviso that when n is O, R^6 and R^7 are not both hydrogen and R^8 and R^9

are not both hydrogen, or R^8 and R^9 may be linked together to form a cycloalkylene ring containing 3 to 6 carbon atoms, and * represents the point of attachment of the R^3 substituent to the remainder of the molecule; and

R^4 is selected from the group consisting of



and



in which R^{10} is hydrogen or methyl, l is 0 or 1, and ** represents the point of attachment of the R^4 substituent to the remainder of the molecule,

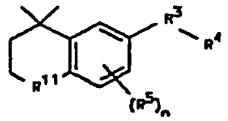
the R^5 are independently selected from the group consisting of lower alkyl and lower alkoxy; and

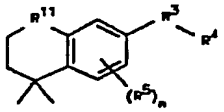
n is 0, 1, 2 or 3,

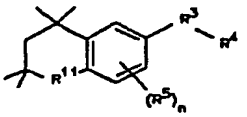
with the proviso that if n is 0, R^3 is other than carbonyl, $*C=CH_2$ or CH_2,

and pharmaceutically acceptable esters, amides and salts of the compound.

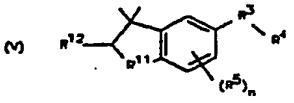
14. The compound of claim 13, having a structure selected from the group consisting of

(II) 

(III) 

(IV) 

and

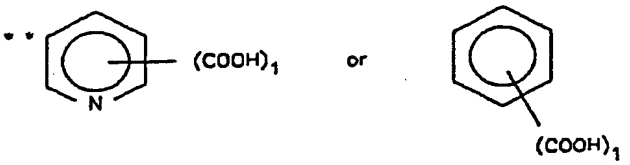
(V) 

where R^{11} is selected from the group consisting of O, S, $(CH_3)_2C$ and CH_2, and R^{12} is hydrogen or methyl.

15. The compound of claim 14, having the structural formula (II).

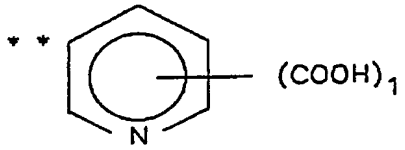
16. The compound of claim 15, wherein n is O.

17. The compound of claim 16, wherein R^4 is

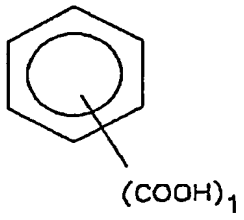


and l is 1.

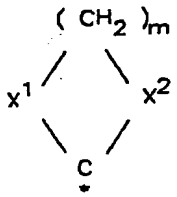
18. The compound of claim 17, wherein R^4 is



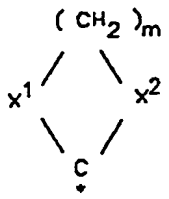
19. The compound of claim 17, wherein R^4 is



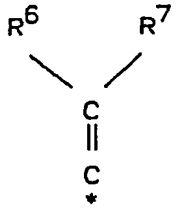
20. The compound of claim 15, wherein R^3 has the structural formula



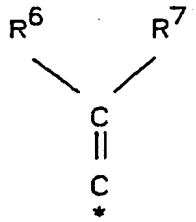
21. The compound of claim 17, wherein R^3 has the structural formula



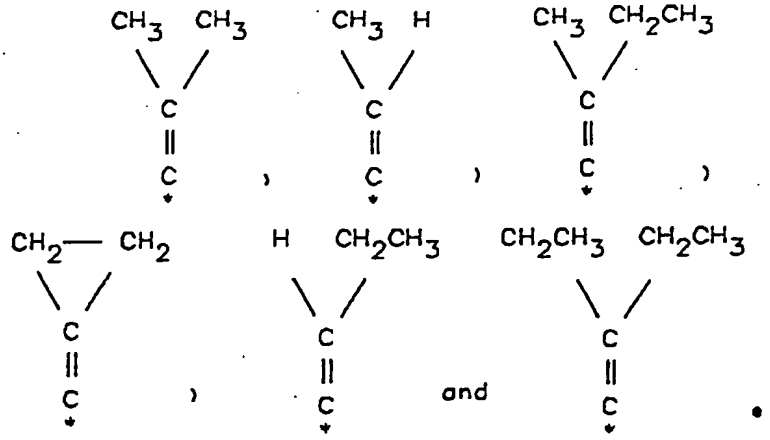
22. The compound of claim 15, wherein R^3 has the structural formula



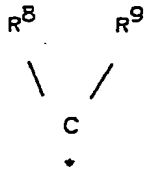
23. The compound of claim 17, wherein R^3 has the structural formula



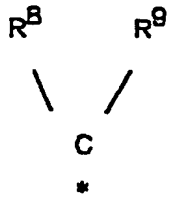
24. The compound of claim 23, wherein R^3 is selected from the group consisting of



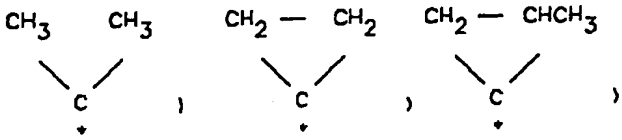
25. The compound of claim 15, wherein R^3 has the structural formula



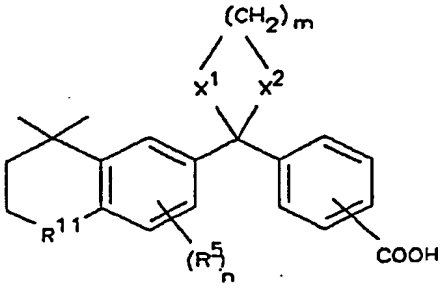
26. The compound of claim 17, wherein R^3 has the structural formula



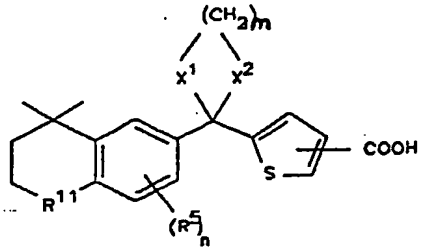
27. The compound of claim 26, wherein R^3 is selected from the group consisting of



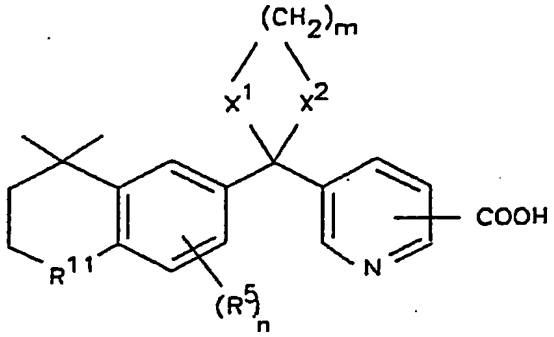
28. The compound of claim 15, having the structural formula



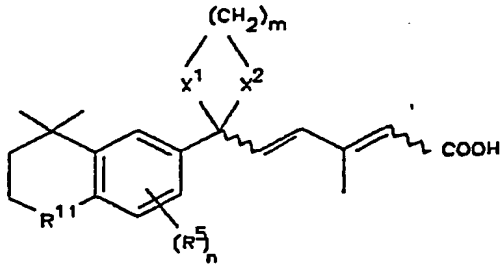
29. The compound of claim 15, having the structural formula



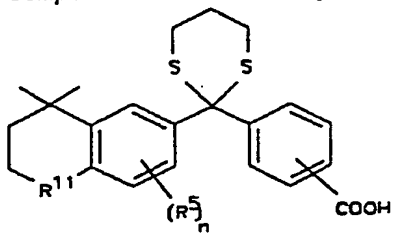
30. The compound of claim 15, having the structural formula



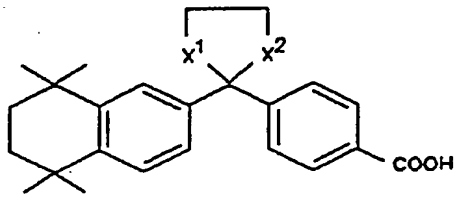
31. The compound of claim 15, having the structural formula



32. The compound of claim 15, having the structural formula

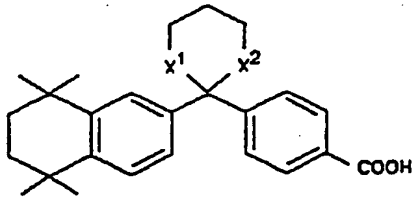


33. The compound of claim 15, having the structural formula



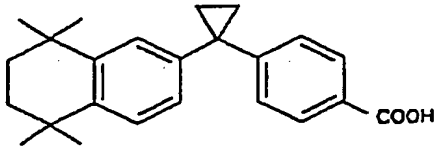
wherein X^1 and X^2 are independently selected from the group consisting of O and S, and the ammonium salt of the compound.

34. The compound of claim 15, having the structural formula

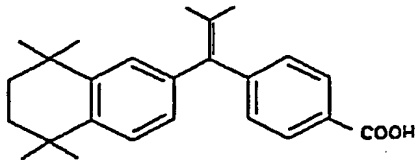


wherein X^1 and X^2 are independently selected from the group consisting of O and S, and the ammonium salt of the compound.

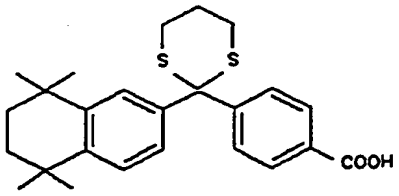
35. The compound of claim 15, having the structural formula



36. The compound of claim 15, having the structural formula

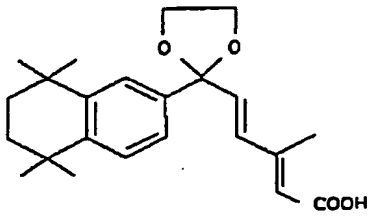


37. The compound of claim 15, having the structural formula



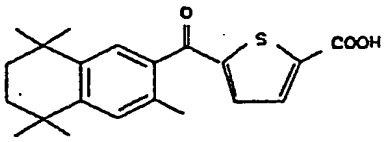
wherein R^1 is O or S.

38. The compound of claim 15, having the structural formula

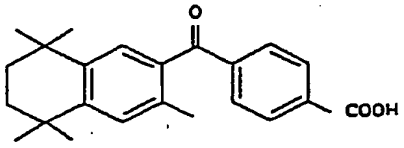


and the ammonium salt of the compound.

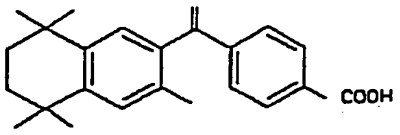
39. The compound of claim 15, having the structural formula



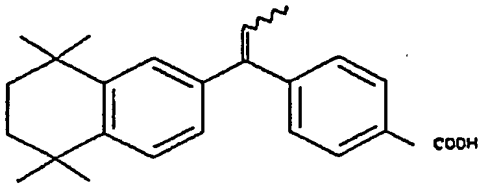
40. The compound of claim 15, having the structural formula



41. The compound of claim 15, having the structural formula



42. The compound of claim 15, having the structural formula



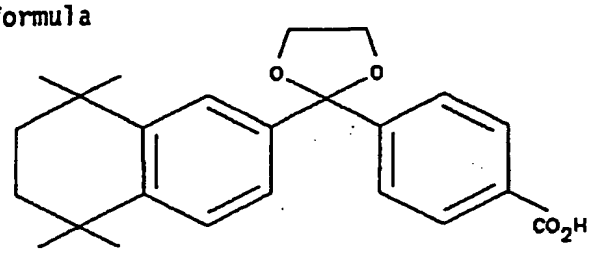
43. A method of inhibiting an activity of a retinoid X receptor heterodimer comprising inducing the formation of a retinoid X receptor homodimer, thereby preventing the retinoid X receptor from forming a heterodimer and preventing the resulting heterodimer activity.

44. The method of claim 43, wherein the activity is the activation or repression of transcription.

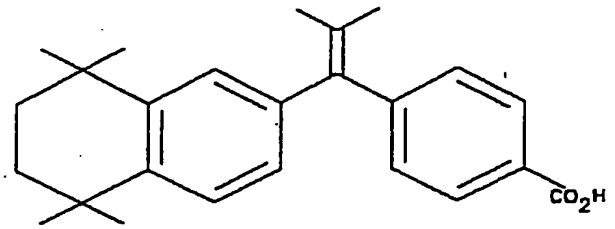
45. The method of claim 43, wherein the retinoid X receptor heterodimer is thyroid hormone receptor and retinoid X receptor.

46. A method of promoting the transcription of a gene activated by a retinoid X receptor homodimer in a cell comprising contacting the cell with an amount of a synthetic compound capable of promoting the formation of retinoid X receptor homodimers.

47. The method of claim 46, wherein the compound has the structural formula



48. The method of claim 46, wherein the compound has the structural formula



49. A method of inhibiting an activity of a retinoid X receptor homodimer comprising preventing the formation of the retinoid X receptor homodimer.

50. The activity of claim 49, wherein the activity is the activation or repression of transcription.

51. A method of inhibiting an activity of a retinoid X receptor homodimer comprising preventing the binding of the retinoid X receptor homodimer to its response element.

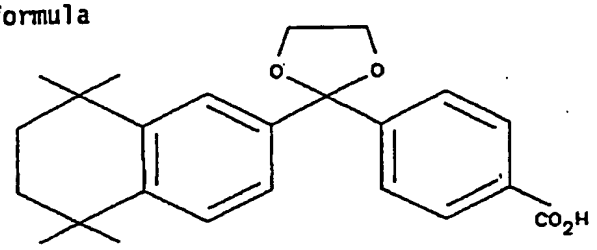
52. The method of claim 51, wherein the activity is the activation or repression of transcription.

53. A method of determining an increased probability of a pathology associated with retinoid X receptor homodimer formation comprising

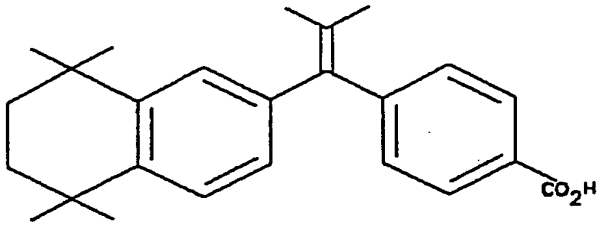
detecting a decrease of retinoid X receptor homodimer formation in the subject when compared to a normal subject.

54. A method of treating a pathology associated with retinoid X receptor homodimers in a subject comprising inducing retinoid X receptor homodimer formation in the subject, thereby increasing the expression of a gene which can be activated by a retinoid X receptor homodimer.

55. The method of claim 54, wherein retinoid X receptor homodimer formation is induced by the addition of a compound having the structural formula



56. The method of claim 54, wherein retinoid X receptor homodimer formation is induced by the addition of a compound having the structural formula



57. The method of claim 54, wherein the pathology is associated with the skin.

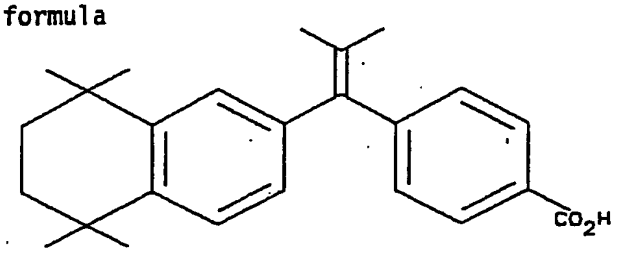
58. The method of claim 54, wherein the skin pathology is selected from the group consisting of acne and psoriasis.

59. The method of claim 54, wherein the pathology is a cancer.

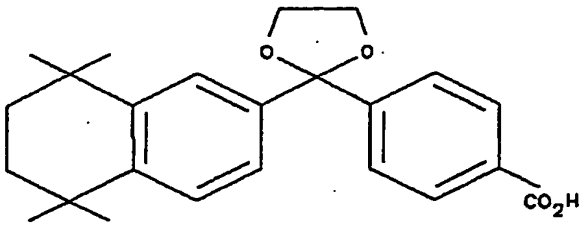
60. The method of claim 54, wherein the gene is the apolipoprotein AI gene.

61. A method of selectively activating retinoid X receptor homodimer formation in a cell comprising adding to the cell a homodimer formation specific ligand, thereby selectively activating the retinoid X receptor homodimer formation in the cell.

62. The method of claim 61, wherein the compound has the structural formula



63. The method of claim 61, wherein the compound has the structural formula



64. A method of promoting retinoid X receptor homodimer formation in a cell comprising the expression of 9-cis-RA in the cell.

65. A pharmaceutical composition for control of cellular processes regulated by retinoic acid, vitamin D, or thyroid hormone, comprising an

effective regulating amount of the compound of claim 13 in combination with a pharmaceutically acceptable carrier.

66. A pharmaceutical composition for control of cellular processes regulated by retinoic acid, vitamin D, or thyroid hormone, comprising an effective regulating amount of the compound of claim 15 in combination with a pharmaceutically acceptable carrier.

67. A method for modulating gene expression, in a subject, by a receptor selected from the group consisting of retinoic acid receptors, retinoid X receptors, vitamin D receptors and thyroid receptors, comprising administering to the subject an effective modulating amount of the compound of claim 13 or a pharmaceutical composition thereof.

68. A method for modulating gene expression, in a subject, by a receptor selected from the group consisting of retinoic acid receptors, retinoid X receptors, vitamin D receptors and thyroid receptors, comprising administering to the subject an effective modulating amount of the compound of claim 15 or a pharmaceutical composition thereof.

69. A method for treating a subject afflicted with a disease caused by malfunction of cell differentiation processes regulated by retinoids, thyroid hormone, or vitamin D, comprising administering to the subject a therapeutically effective amount of the compound of claim 13 or a pharmaceutical composition thereof.

70. A method for treating a subject afflicted with a disease caused by malfunction of cell differentiation processes regulated by retinoids, thyroid hormone, or vitamin D, comprising administering to the subject a therapeutically effective amount of the compound of claim 15 or a pharmaceutical composition thereof.

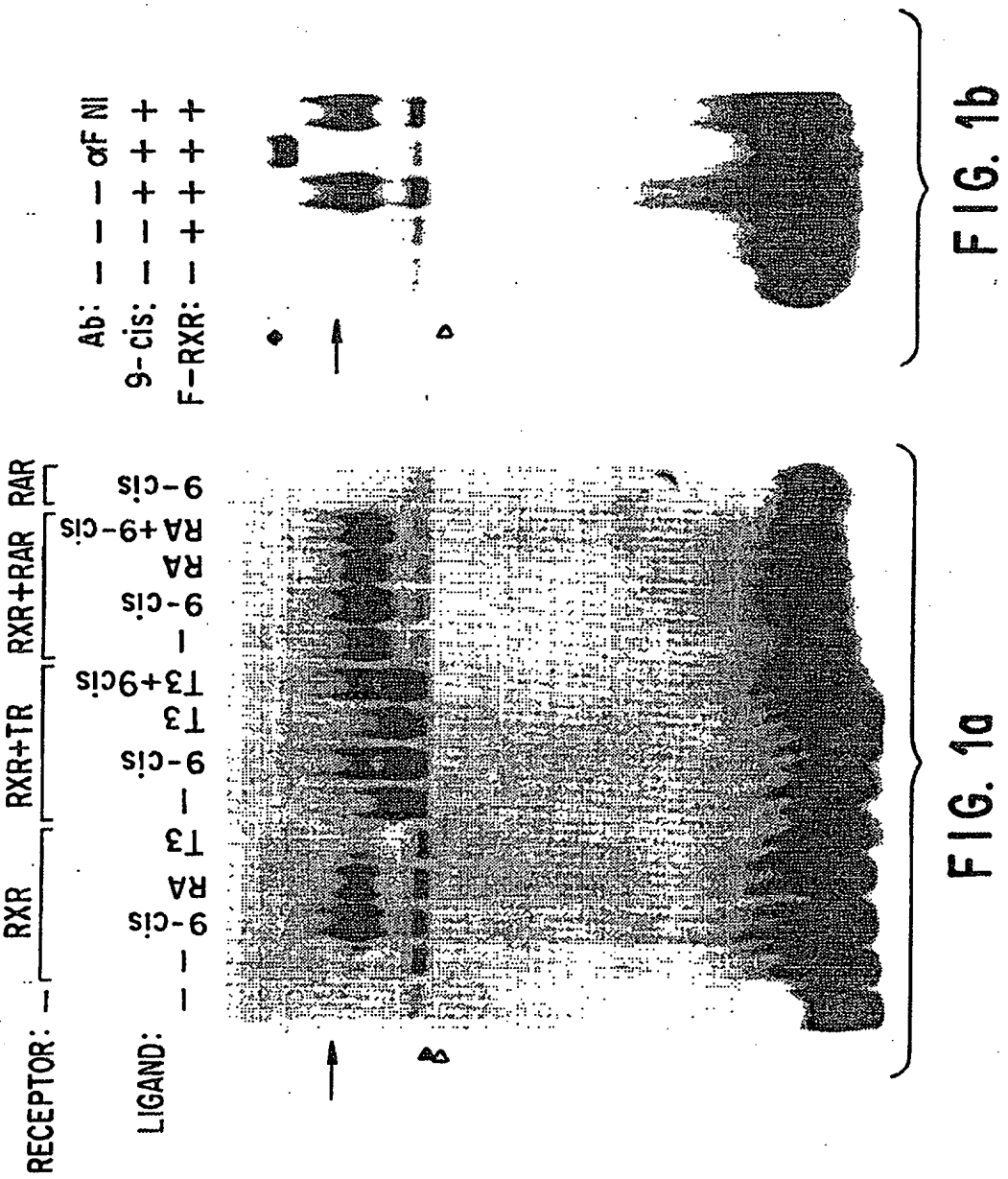

Ab: - - - αF NI + +
9-cis: - - + + + +
F-RXR: + + + + - -
RECEPTOR: - RXR RXR+TR RXR+RAR RAR
LIGAND: - - 9-cis RA T3 - 9-cis T3 T3+9cis - 9-cis RA RA+9-cis 9-cis

FIG. 1b

FIG. 1a

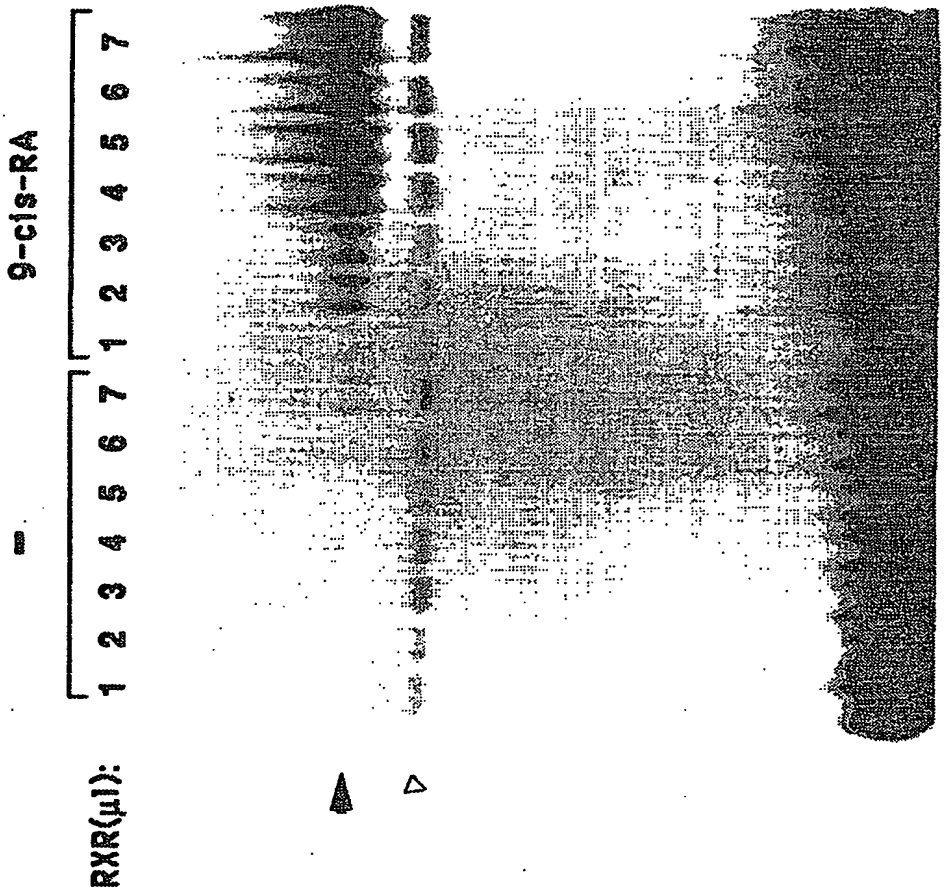


FIG. 2a

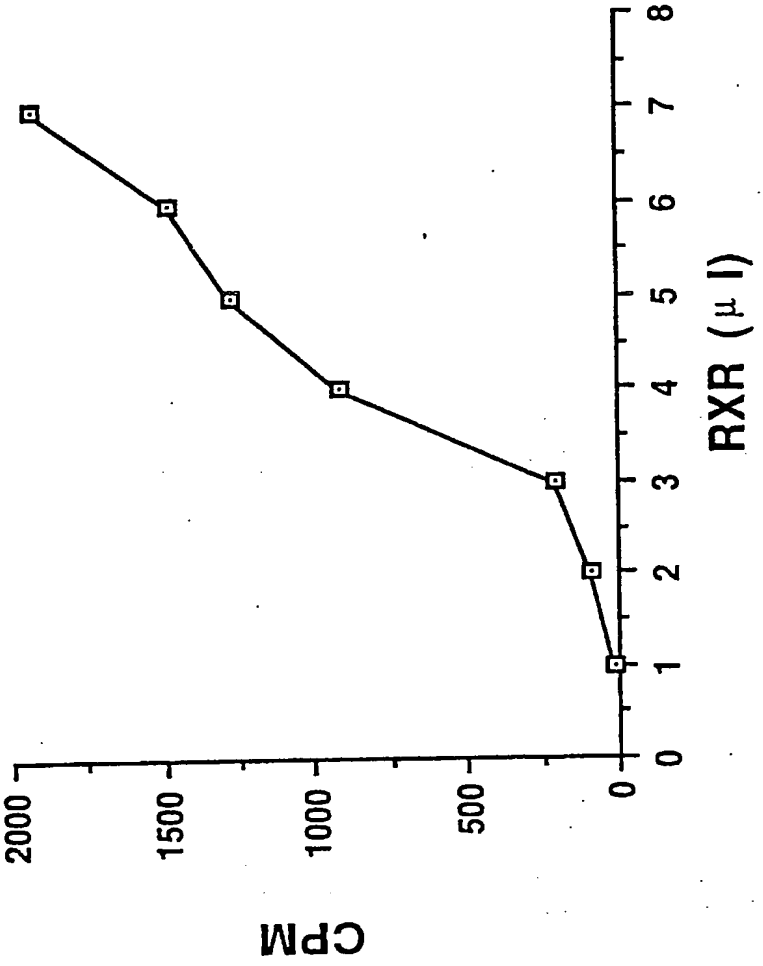


FIG. 2b

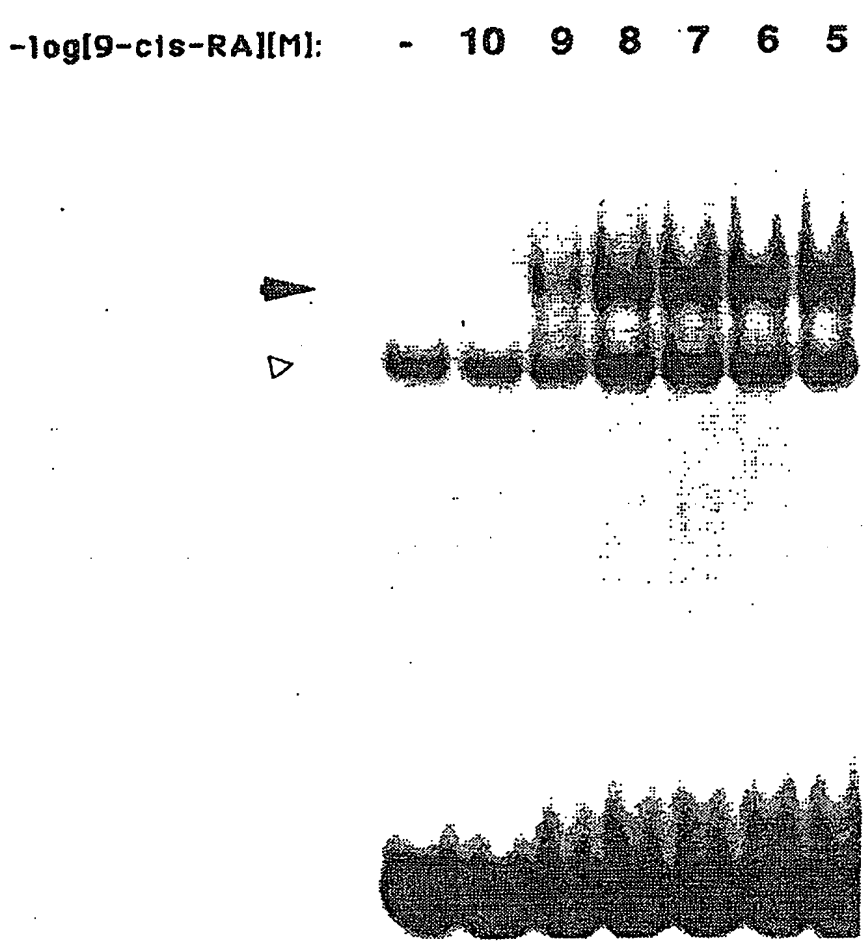


FIG. 2c

TREpal	gatcTCAGGTCATGACCTGAgatc ctagAGTCCAGTACTGGACTctag
ApoAI-RARE	gatcAGGGCAGGGGTCAAGGGTTCAGTgatc ctagTCCCGTCCCCAGTTCCCAAGTCActag
CRBPII-RARE	gatcCAGGTCACAGGTCACAGGTCACAGTTCAAgatc ctagGTCCAGTGTCCAGTGTCCAGTGTCAAGTTctag
βRARE	gatctGTAGGGTTCACCGAAAGTTCACTCagatc ctagaCATCCCAAGTGGCTTTCAAGTGAGtctag
CRBPI-RARE	gatccAGGTCAAAAAGTCAGgatc ctaggTCCAGTTTTTCAGTCctag
MHC-TRE	gatcCTGGAGGTGACAGGAGGACAGCgatc ctagGACCTCCACTGTCCTCCTGTCGctag
ME-TRE	gatcCAGGACGTTGGGGTTAGGGGAGGACAGTGGgatc ctagGTCCTGCAACCCCAATCCCCTCCTGTCACCctag
DR-4	gatcTCAGGTCATCTCAGGTCAgatc ctagAGTCCAGTAGAGTCCAGTctag
DR-5	gatcTCAGGTCATCCTCAGGTCAgatc ctagAGTCCAGTAGGAGTCCAGTctag
ERE	gatcTCAGGTCACTGTGACCTGAgatc ctagAGTCCAGTGACACTGGACTctag

FIG. 3a

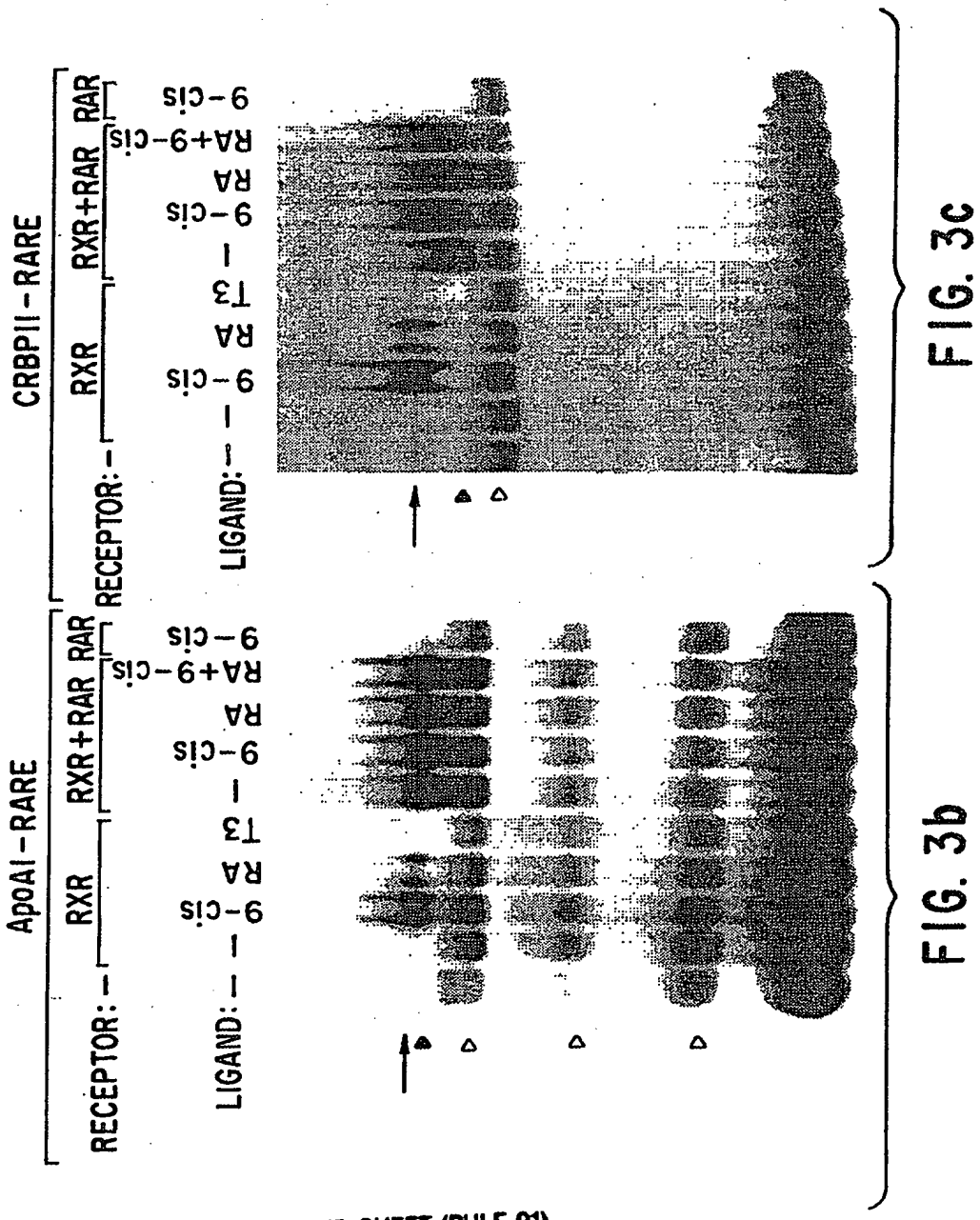

CRBPII-RARE
RECEPTOR: - RXR RXR+RAR RAR
LIGAND: - 9-cis RA T3 9-cis RA+9-cis 9-cis
ApoAI-RARE
RECEPTOR: - RXR RXR+RAR RAR
LIGAND: - 9-cis RA T3 9-cis RA+9-cis 9-cis

FIG. 3c

FIG. 3b

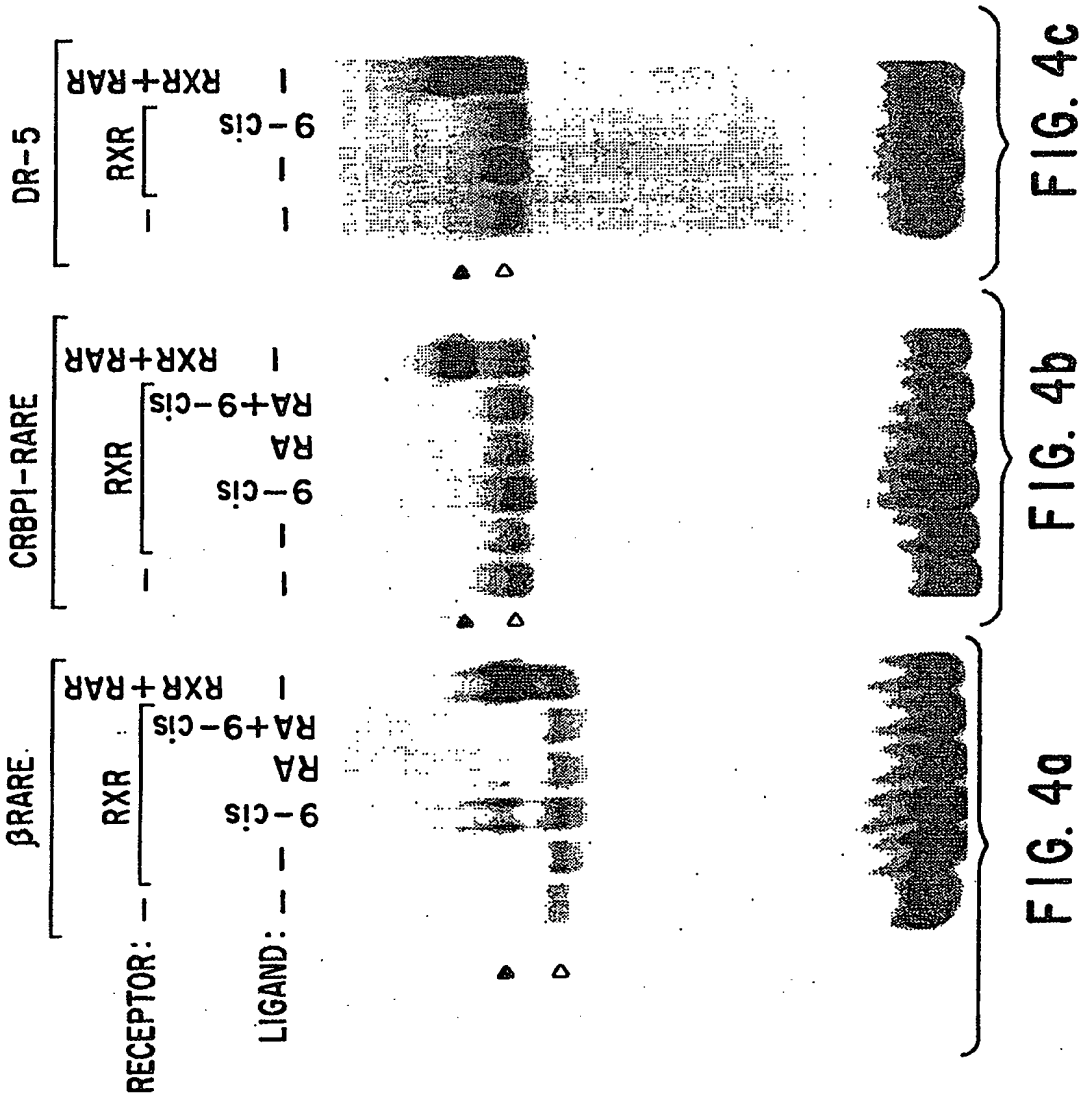
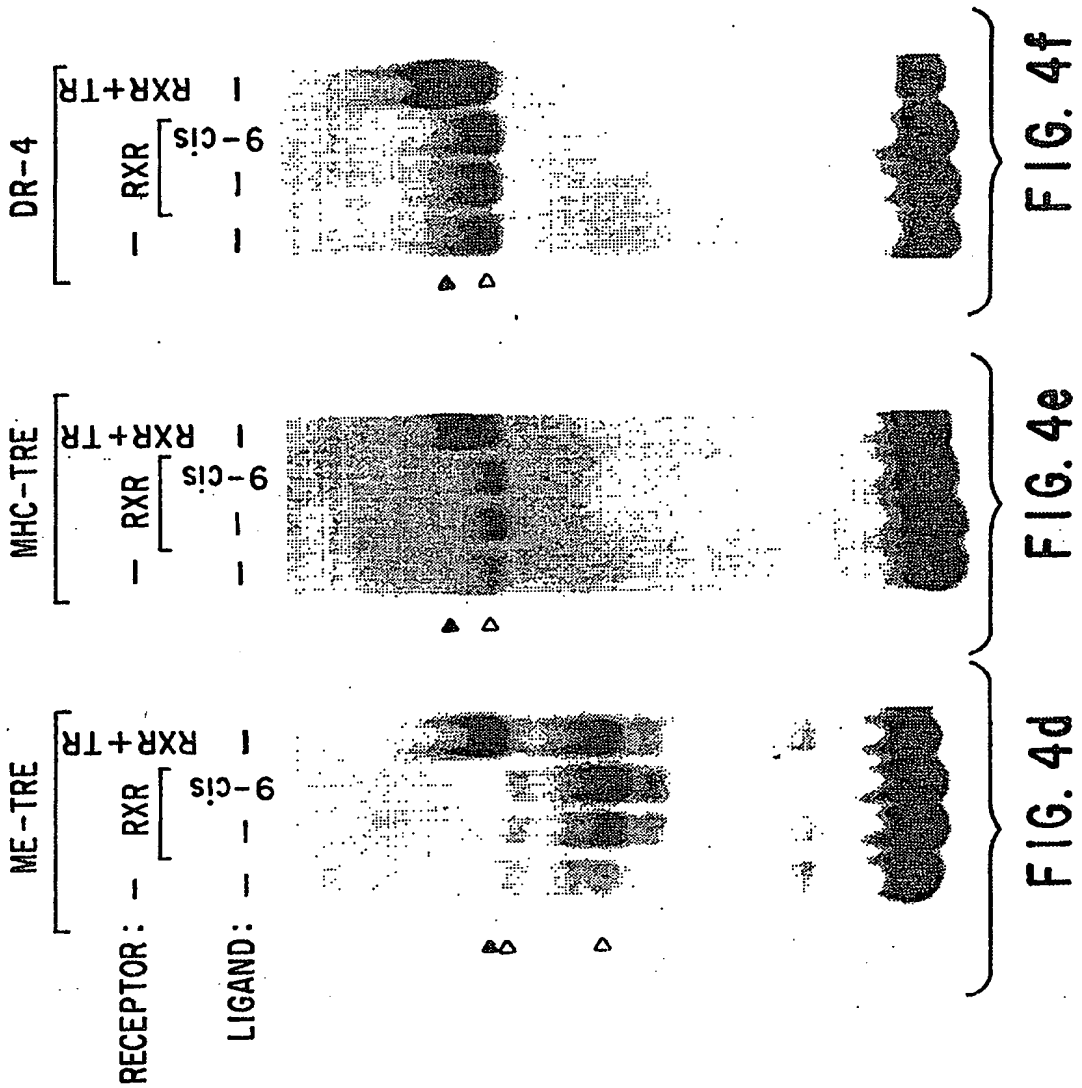


FIG. 4a

FIG. 4b

FIG. 4c


DR-4
MHC-TRE
ME-TRE
RXR+TR
RXR
9-cis
RECEPTOR:
LIGAND:
FIG. 4f
FIG. 4e
FIG. 4d

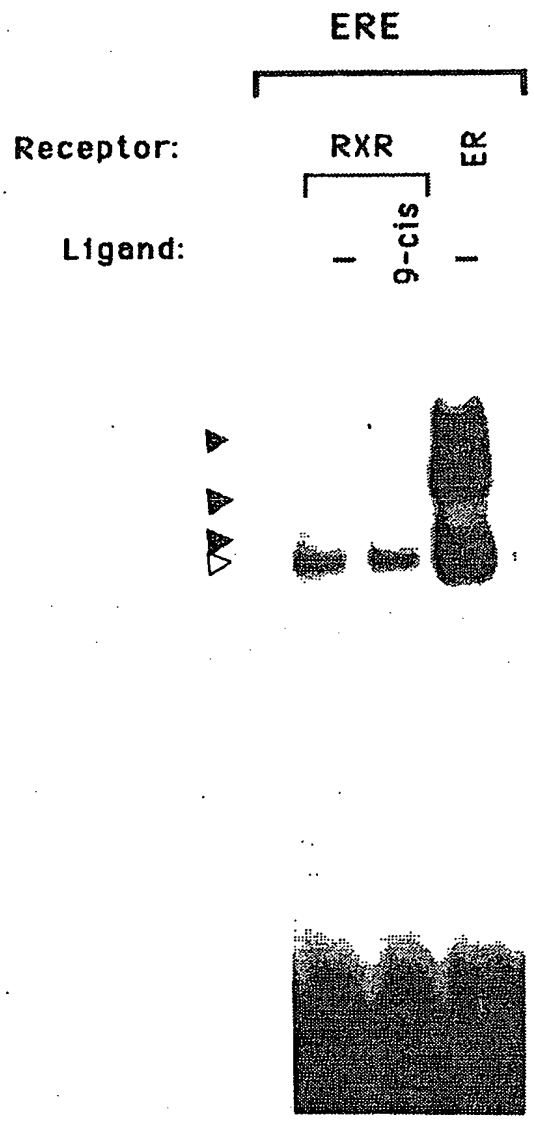


FIG. 4g

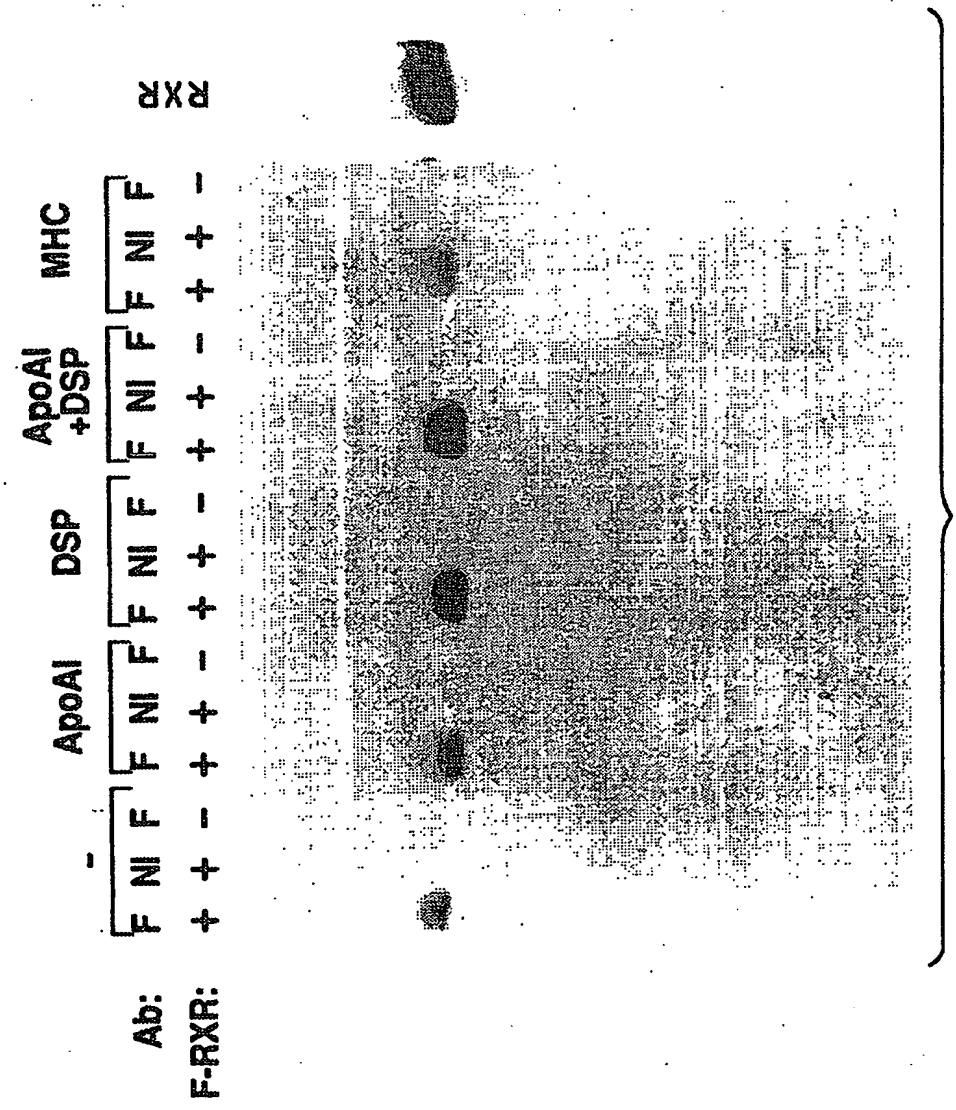


FIG. 5

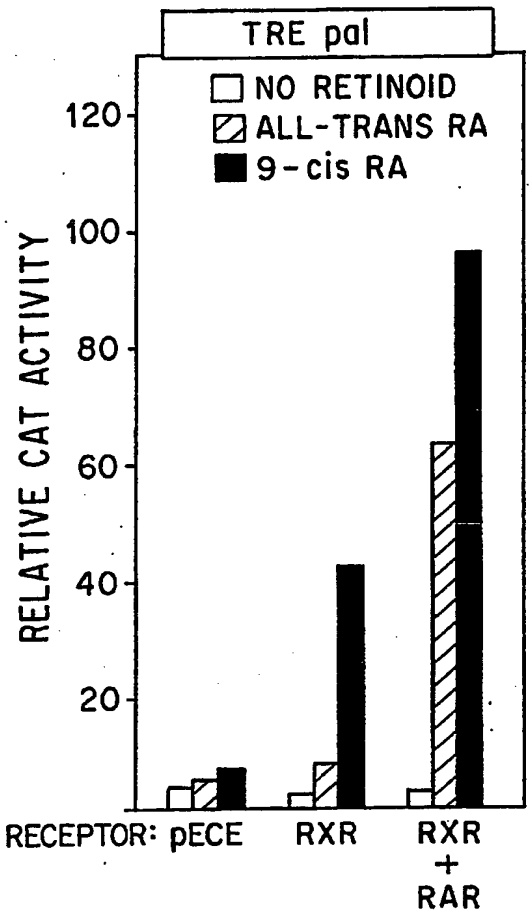

TRE pal
NO RETINOID
ALL-TRANS RA
9-cis RA
RELATIVE CAT ACTIVITY
120
100
80
60
40
20
RECEPTOR: pECE
RXR
RXR + RAR

FIG. 6a

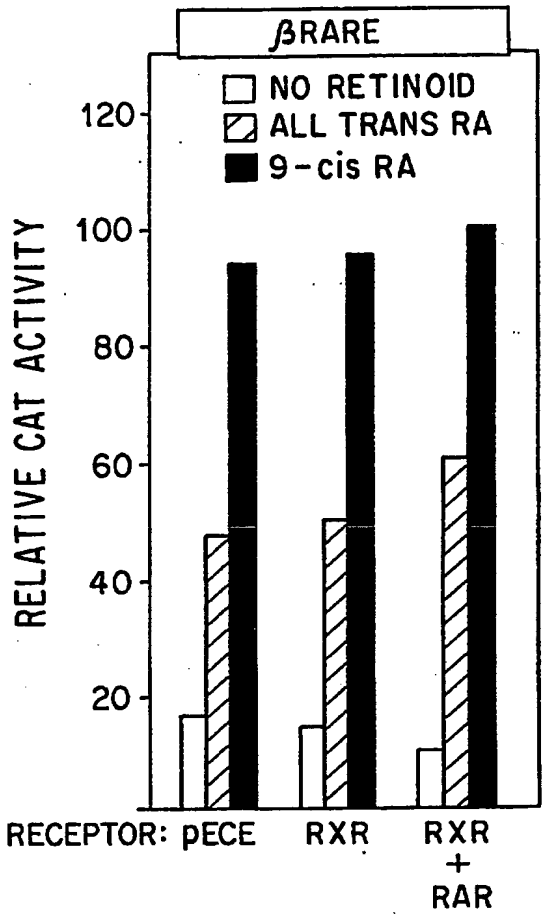

βRARE
NO RETINOID
ALL TRANS RA
9-cis RA
RELATIVE CAT ACTIVITY
120
100
80
60
40
20
RECEPTOR: pECE
RXR
RXR + RAR

FIG. 6b

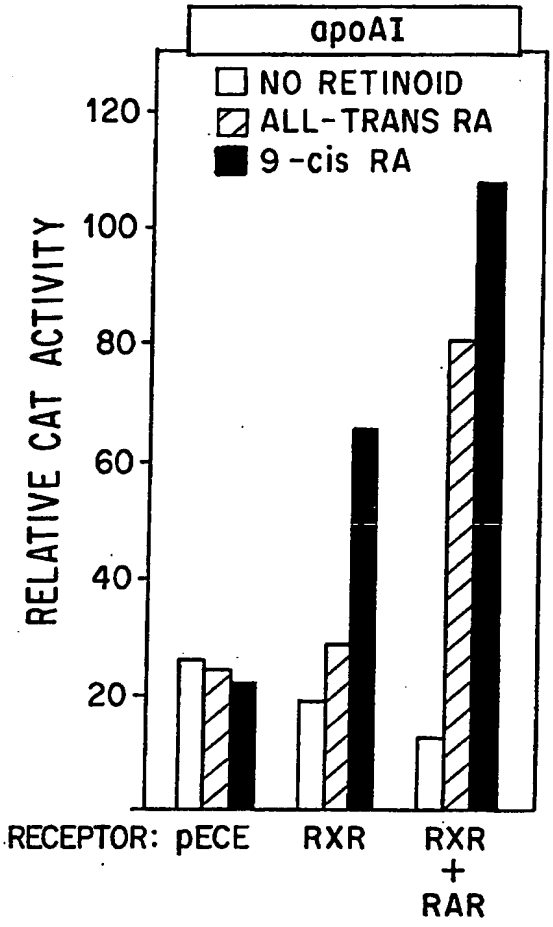

apoAI
NO RETINOID
ALL-TRANS RA
9-cis RA
RELATIVE CAT ACTIVITY
120
100
80
60
40
20
RECEPTOR: pECE
RXR
RXR + RAR

FIG. 6c

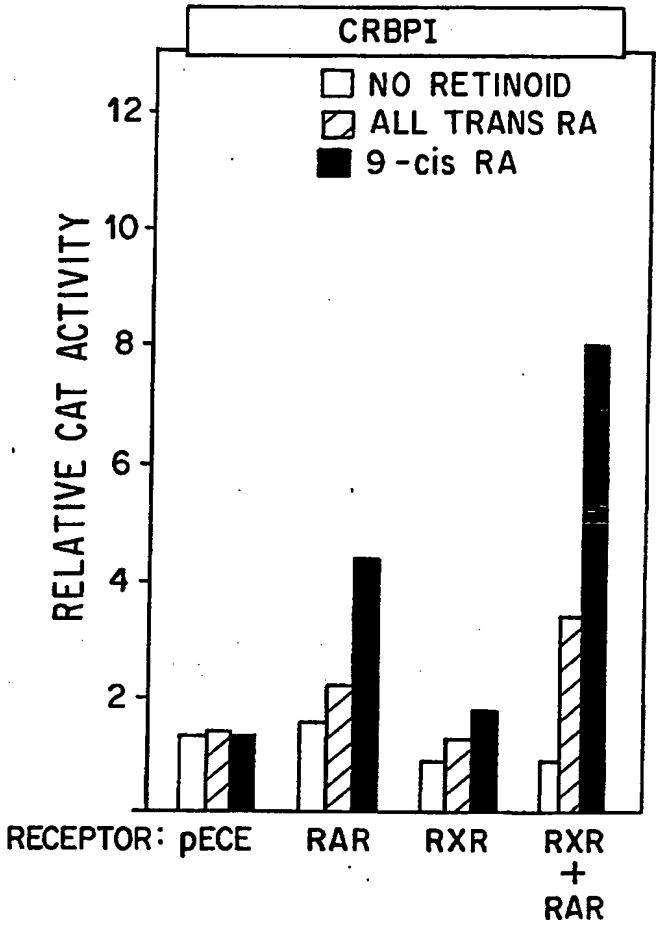

CRBPI
NO RETINOID
ALL TRANS RA
9-cis RA
RELATIVE CAT ACTIVITY
12
10
8
6
4
2
RECEPTOR: pECE
RAR
RXR
RXR + RAR

FIG. 6d

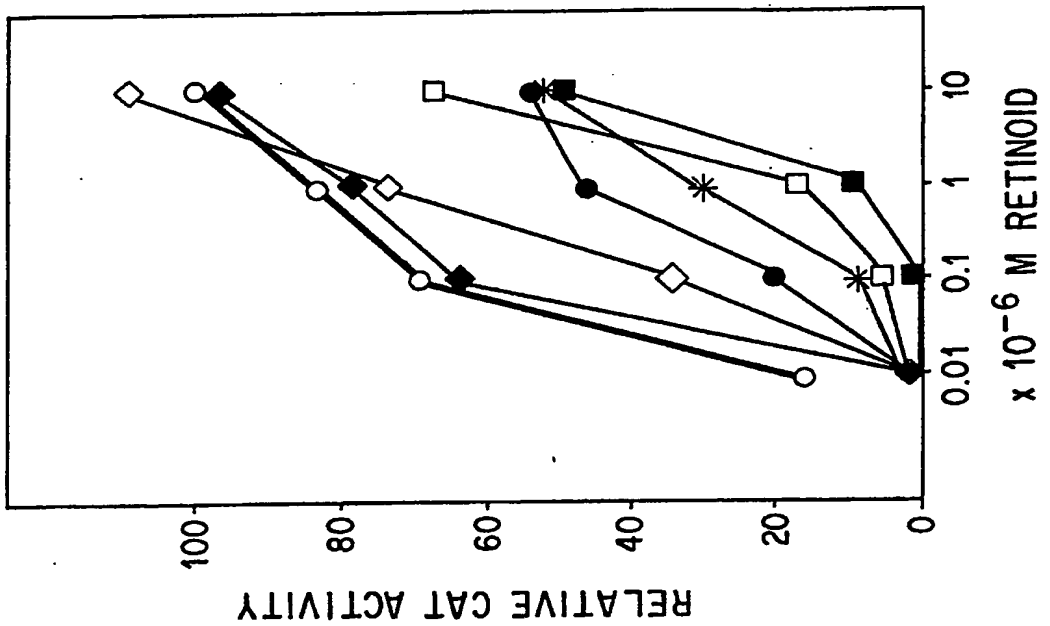

RELATIVE CAT ACTIVITY
0
20
40
60
80
100
0.01
0.1
1
10
x 10⁻⁶ M RETINOID

FIG. 7b

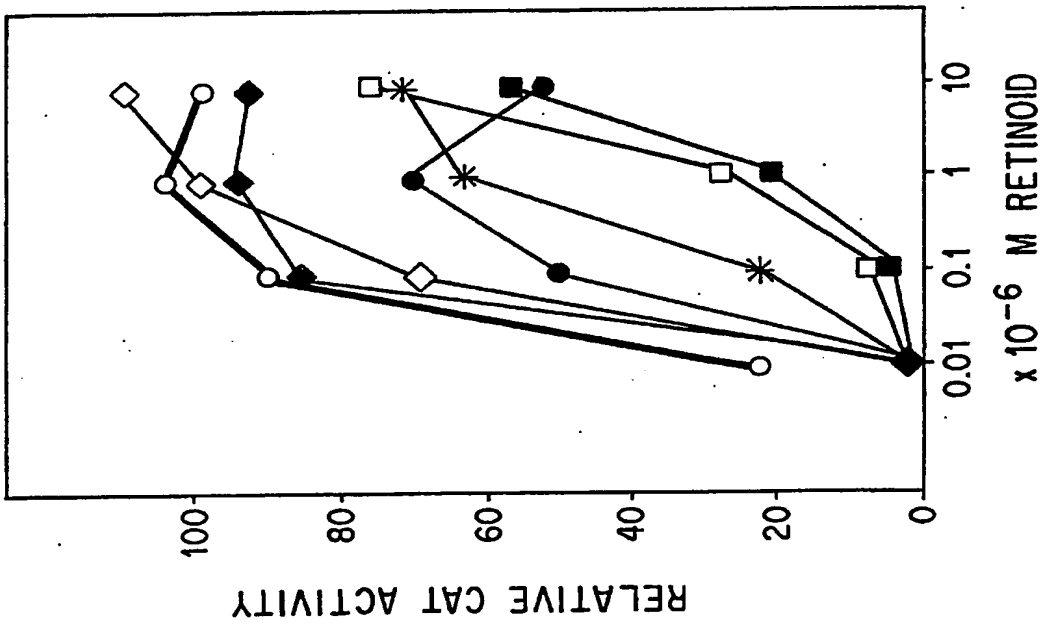

RELATIVE CAT ACTIVITY
0
20
40
60
80
100
0.01
0.1
1
10
x 10⁻⁶ M RETINOID

FIG. 7a